Int. J. Dev. Biol. 34: 149-156 (1990)

From presumptive ectoderm to neural cells in an amphibian

ANNE-MARIE DUPRAT*, JEAN-PIERRE SAINT-JEANNET, FABIENNE PITUELLO, SONG HUANG, SAÏDA BOUDANNAOUI, PAULETTE KAN and LYDIE GUALANDRIS

Centre de Biologie du Développement, URA-CNRS 675, affiliée à l'INSERM, Université Paul Sabatier, Toulouse, France

ABSTRACT As an immediate consequence of neural induction during gastrulation, some neuroectodermal cells acquire the ability to develop a number of specific neuronal and astroglial features, without requiring subsequent chordamesodermal cues. Thus, cholinergic, dopaminergic, noradrenergic, gabaergic, somatostatinergic, enkephalinergic, etc. traits are expressed in cultures of neural plate and neural fold isolated from amphibian late gastrulae immediately after induction and cultured in a defined medium. These results strongly suggest that at the late gastrula stage, the neural precursor population does not yet constitute a homogeneous set of cells. It was of interest to know the origin of this heterogeneity. Is it a direct result of the process of neural induction itself, stochastic phenomena being involved or not at the cellular level, or does it reflect a pre-existing heterogeneity in the presumptive ectoderm? At the early gastrula state, presumptive ectoderm can be neuralized consecutively to its dissociation into single cells. Using this experimental model, we have demonstrated by means of immunological probes that neuralized presumptive ectodermal cells, without any intervention of the chordamesoderm (natural inducing tissue), can develop autonomously into glial and neuronal lineages. These data suggest the existence of diverse predispositions of presumptive ectodermal cells. Competent ectoderm seems to be a heterogeneous structure with cells presenting distinct neural predispositions that can emerge as a consequence of a permissive inductive signal without real specificity (such as a target tissue dissociation). Moreover, such a differentiated neuronal population includes neurons of the GABAergic and enkephalinergic phenotypes but not of the cholinergic, catecholaminergic, somatostatinergic, etc. phenotypes. These data show that the developmental program of ectodermal cells induced without interaction with the developmental program of ectodermal cells induced whitout interaction with the chordamesoderm appears restricted compared to the naturally induced ectoderm. Experiments are now under way to analyze such sequential neural events.

KEY WORDS: *neural induction, neurogenesis, neurons, glial cells, amphibian*

Introduction

In the embryo, processes by which embryonic cells become differentiated depend on complex tissue interactions and cell microenvironmental cues and imply intracellular molecular mechanisms leading to the expression and regulation of genes specific for each differentiated cell phenotype.

One of the main questions in embryology, therefore, concerns the acquisition (during embryonic induction) and expression of a particular commitment by some cells, at precise stages in the ontogeny of the embryo. Such a spatial and temporal succession of programmed steps is the result of very complex interactions between genetic and epigenetic factors.

With regard to neural induction, which remains one of the oldest and nevertheless still unsolved problems (cf. Duprat *et al.*, 1987; Gurdon, 1987; Holtfreter, 1988; Saxén, 1989), we (Duprat *et al.*, 1982) felt that a new conceptual framework was needed for

Abbreviations used in this paper: ACh, acetylcholine; AChE, acetylcholinesterase; CAT, choline acetyltransferase; CM, chordamesoderm; CNS, central nervous system; ECM, extracellular matrix; GABA, gamma aminobutyric acid; GFAp, glial fibrillary acidic protein; HEDAF, 5-(N-hexadecanoyl)-aminofluorescein; isoONPA, tetra-isopropyl-pyrophosphoramide; NF, neural fold; NP, neural plate; PNS, peripheral nervous system.

***Address for reprints**: Centre de Biologie du Développement, URA-CNRS 675, affiliée à l'INSERM, Université Paul-Sabatier, 118 route de Narbonne, F-31062 Toulouse cedex, France

0214-6282/90

analyzing the process of neural determination, based more on the crucial role played by the competent target cells than on the inducer itself. Many different tissues as well as many chemically unrelated substances were found, which, when applied to the competent presumptive ectoderm, had a neuralizing effect. Whatever the mechanisms of action of the numerous inducing factors known until now (cf. for review Saxén and Toivonen, 1962; Nieuwkoop *et al.*, 1985) it is quite possible that the competent target tissue itself contains the key to the problem. What the neuralizing factors so far studied seem to have in common is the capability to initiate at the target tissue level the same signal, setting in motion the molecular machinery specific for neural commitment.

Transmission of the inductive signal

During gastrulation, the inductive process is most probably a recognition of some external signal which is perceived and converted by neural target cells at the plasma membrane level (Tiedemann and Born, 1978; Duprat *et al.*, 1982; Gualandris *et al.*, 1985; Born *et al.*, 1986; Otte *et al.*, 1988, 1989). We have previously reported that neural induction depends critically on the molecular structure of the competent target tissue membrane. Using lectin probes, we showed that lectin binding on the surface of living target cells leads to a reorganization of membrane glycoconjugates and that this rearrangement strongly inhibits the inductive process consecutive to the effect of the natural inducer (chordamesoderm). The inhibitory effect is reversed and disappears with time (about 20 hours) as a result of normal membrane glycoconjugates turnover. We demonstrated that this inhibiting effect did not appear to be due to crowding on the cell surface by lectins, since binding of these succinylated-lectins had no inhibitory effect (these succinylated lectins did not evoke the rearrangement of their binding glycoconjugates) (Gualandris *et al.*, 1983, 1985). These results indicated that membrane glycoconjugates of the target cells play a role in the onset of the molecular events for neural induction. We also demonstrated that this structural modification affects the reception but not the subsequent transmission of the inductive signal to the intracellular machinery and the expression of the neural inductive (or permissive) information (Gualandris and Duprat, 1987).

Thus, we conclude that a particular organization of plasma membrane glycoconjugates of the competent target ectodermal cells appears to be required for the reception of the natural neural inducing signal and/or the initiation of neural induction. The neural commitment of induced cells is not affected by such a structural glycoconjugate rearrangement immediately after induction.

The possible involvement of a specific membrane glycan receptor in the binding of the external natural inductive signal has been examined. Many different glycoconjugated molecules (N-acetyl-D-galactosamine, α-D-galactose and derivatives, N-acetyl-D-glucosamine, α-D-glucose, etc.) would have to be involved (Gualandris *et al.*, 1985). We showed that the close implication of such specific receptor molecules in neural induction is so far hypothetical and remains to be demonstrated. We are inclined to the view that it is particular physicochemical and electrophysiological properties of the competent target membrane that could play an important role in the initiation of this inductive process. Experiments are now being performed in this field. Using the "fluorescence recovery after photo-bleaching" technique, we found that the lateral diffusion rate of a lipophilic probe (HEDAF) inserted into the plasma membrane of presumptive ectoderm was altered from early blastula (non competent) to early gastrula (competent) and to neurula (induced). These experiments indicated a decrease in the membrane fluidity between non-competent and competent cells (Dupou *et al.*, 1987).

Lectins are a particular family of proteins which bind to carbohydrate residues in glycoproteins or other glycoconjugates by interacting with specific configurations of oligosaccharides. Biochemical experiments are now being conducted to discover the part played by the oligosaccharide moiety of the target glycoconjugates in this interactive process between external neural signal and target tissue.

In previous studies (Duprat and Gualandris, 1984a) we also showed that the extensive network of extracellular matrix (ECM) covering the internal surface of the target tissue at the gastrula stage is not involved in the process of neural induction itself. Both the inner (with ECM) and the outer (without ECM) surfaces of the competent presumptive ectoderm can be induced *in vitro* by association with chordamesoderm (natural inducer). Moreover, binding of anti-fibronectin antibodies to ectodermal tissue prior to its association with the inducer did not inhibit neural induction. During gastrulation, this ECM is essentially implicated in cell migration and morphogenetic movements (Boucaut *et al.*, 1984).

The knowledge accumulated over the space of 60 years (!) on the highly recalcitrant problem of neural induction is extensive but insufficient; one can however share Holtfreter's "optimistic" conclusion (1988): "The fundamental principles underlying (neural) embryonic induction have remained obscure. However, (...) animal development is predictable, repeating itself in every generation. We can be sure that the eggs of frogs and salamanders will always develop according to the very same blueprint that has guided development of their ancestors for eons. How fortunate for the future generation of embryologists. They will still have the opportunity to study and solve the old riddles of development that have defied scientists up till now".

It is clear that a multidisciplinary research effort is required to unravel this problem. The wide array of modern methods, including genetic, molecular, electrophysiological, biochemical and cellular techniques shows great promise due to their complementarity and exquisite sensitivity.

The molecular understanding of competence will probably be an important key to this embryological problem.

Neural properties acquired by the target tissue during neural induction

In the dorsal part of the gastrulated amphibian embryo, two different territories appear as an immediate consequence of neural induction: the neural plate (NP) and the neural fold (NF), which are at the origin of the central and peripheral nervous system (CNS and PNS), respectively .

In order to evaluate the differentiation capacities of neural precursor cells just after neural induction occurs, we isolated NP and/or NF from further embryonic environmental cues, dissociated the cells and cultured them in a defined saline medium (Barth and Barth, 1959) (Fig. 1). These cells differentiate among other cell types (Duprat *et al.*, 1966, 1984b) into morphologically recognizable neurons developing highly specific neuronal traits such as neurofilament polypeptides, tetanus toxin binding sites (Duprat *et al.*, 1986) and N-CAM (Saint-Jeannet *et al.*, 1989a). We also showed changes in cell surface carbohydrates with respect to neuronal

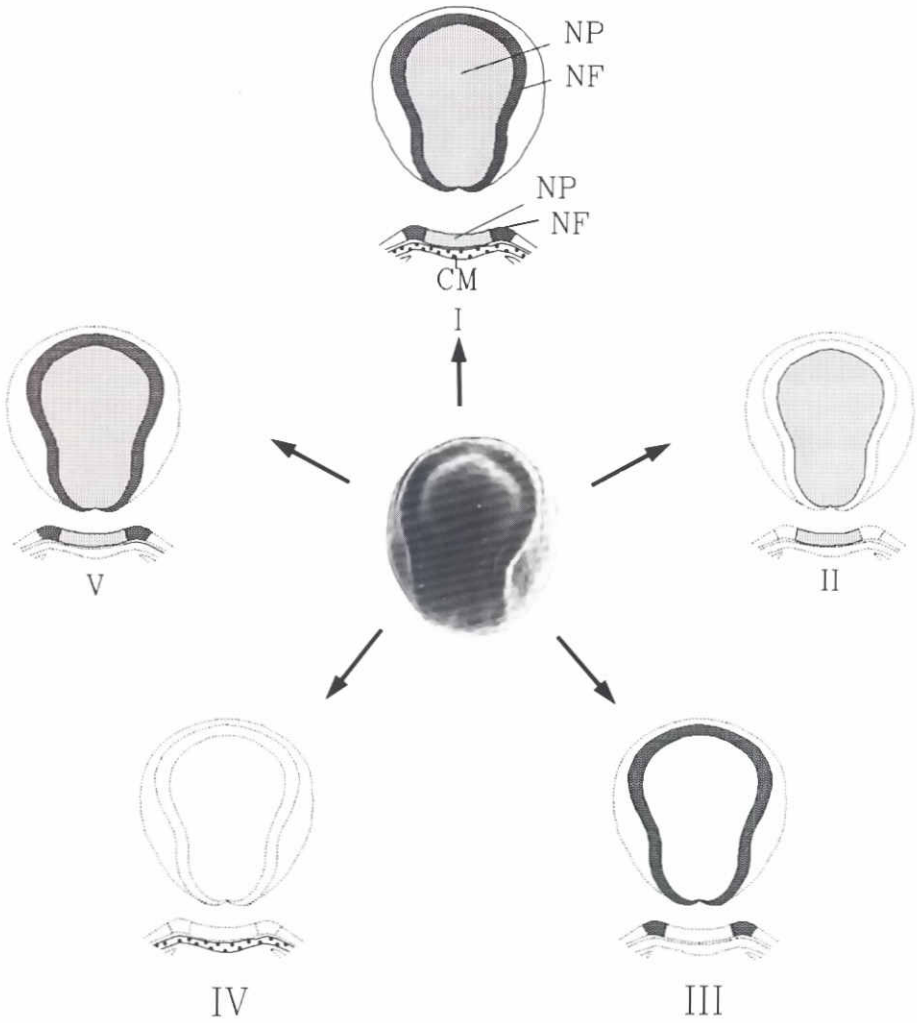

Fig. 1. Procedure for cell cultures. **(I)** *Co-cultures: NP + NF + CM. Neural plate + neural fold cells were co-cultured with chordamesodermal cells.* **(II)** *Isolated neural plate cells (NP).* **(III)** *Isolated neural fold cells (NF).* **(IV)** *Isolated chordamesodermal cells (CM).* **(V)** *Neurectodermal cells (NP + NF).*

differentiation (Duprat *et al.*, 1985a). Distinctive adhesive properties of the neuronal NP and NF precursor cells were one of the earliest events observed *in vitro* (Duprat *et al.*, 1984b, 1985b).

These results indicated that, starting just with committed neurectoderm, phenotypically mature neurons can be obtained *in vitro*, without any further chordamesodermal influence.

It was of interest to know whether neuronal cells acquired, as a direct consequence of neural induction, the capacity to develop neurotransmitter phenotypes.

The development of acetylcholine (ACh) biosynthesis has now been demonstrated for the first time. ACh production increased linearly with time up to 14 days, when neurectodermal cells were co-cultured with chordamesodermal cells (Fig. 1, I); it remained constant and low in neural plate cell cultures (Fig. 1, II) and was absent in neural fold cell cultures (Fig. 1, III) (Duprat *et al.*, 1985b, 1985c). The kinetics of appearance and of activity of the two specific enzymes involved in the cholinergic metabolism: choline acetyltransferase (CAT), the specific enzyme of ACh synthesis, and acetylcholinesterase (AChE) enzyme for ACh degradation, were analyzed. In addition the aim of this study was to focus on the initial expression of different molecular isoforms of AChE in neural plate and in neural fold cells differentiated *in vitro* (Duprat *et al.*, 1990).

CAT and AChE were not detected in neurectodermal cells at the late gastrula stage. The significant appearance of these two enzymes was concomitant with the neuronal differentiation as assessed by morphological criteria and specific marker expression.

CAT and AChE activity was significantly detected in 4-day old co-cultures of neurectodermal cells associated with chordamesodermal cells, and linearly increased with time. Hence it is possible that a single factor regulates the expression of these two proteins in early neuroblasts.

Differences in the patterns of expression of both enzymes were found between NP and NF cultures. In both types of culture, clear neuronal differentiation was observed but only NP cells synthesized significant amounts of cholinergic-related enzymes.

On the basis of the presence or absence of enzyme activity, it is tempting to speculate on the commitment of the neuronal precursor cells from NP and from NF areas at the late gastrula stage, these cells being at the origin of the CNS and the PNS, respectively. Clearly, there are differences a few hours after neural induction between NP and NF cells – similar data were already found by comparing the respective aggregation behavior of NP and NF neuroblasts (Duprat *et al.*, 1984b). In NP area at the early neurula stage, a neuronal precursor population seemed already committed towards the cholinergic pathway and could express this trait when isolated in culture. In contrast, NF cells either were not yet

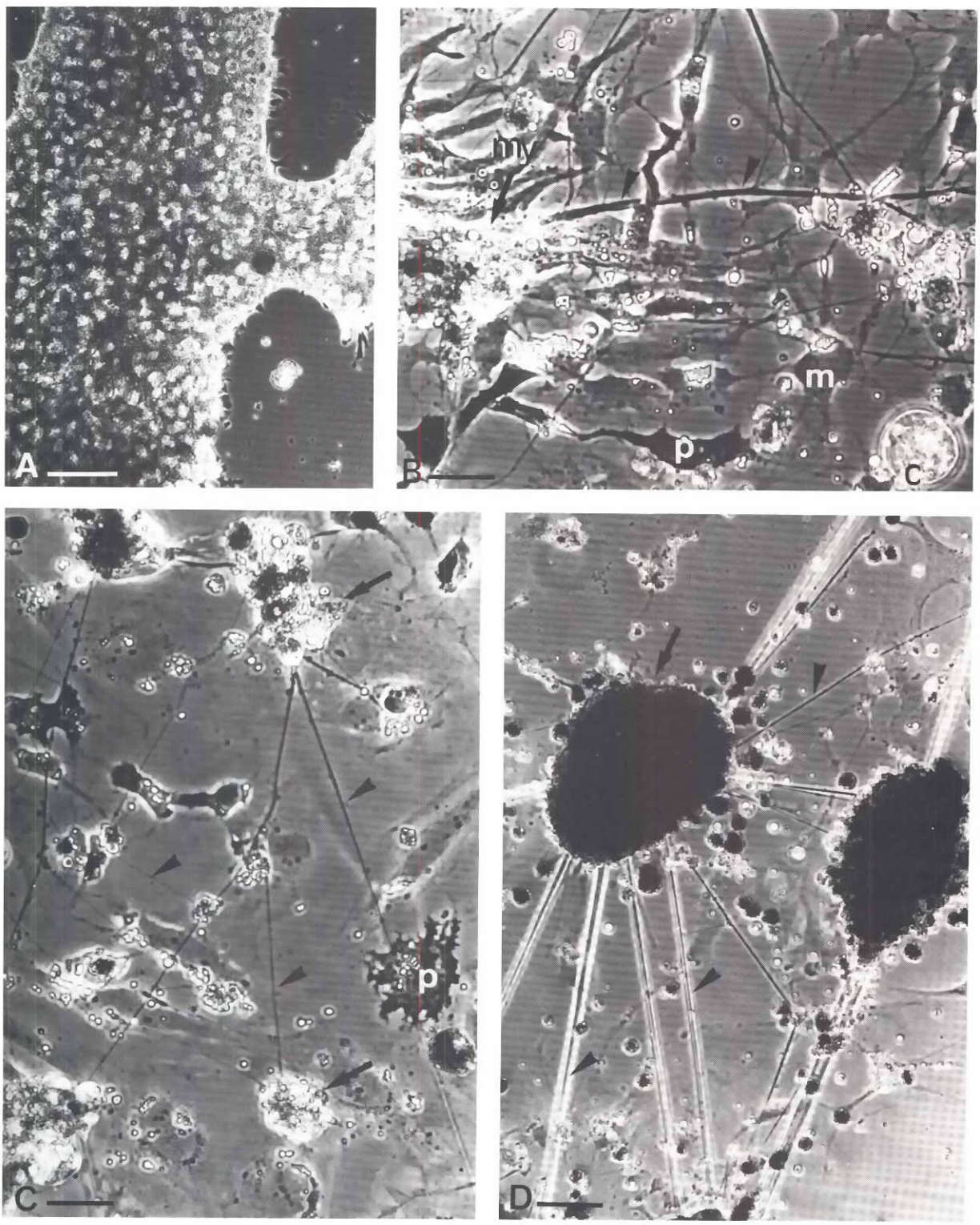

Fig. 2. Phase contrast micrographs. **(A)** *Isolated cells from non-induced ectoderm cultured for 3 days: strong reaggregation and typical epidermal sheet. Bar = 100 μm.* **(B)** *Co-culture: isolated cells from NP + NF + CM cultured up to 12 days. Presence of neurites (arrowheads) pigment cells (p), chordal cell (c), mesenchymal cells (m), myoblasts (my). Bar= 50 μm.* **(C)** *Culture from isolated neural fold cells, 12-day-old culture: dispersed neurons with thin neurites (arrowheads). Bar= 50 μm.* **(D)** *Culture from isolated neural plate cells, 12-day-old culture: neurons reaggregated strongly (arrow), thick fascicules of neurites (arrowheads). Bar= 100 μm.*

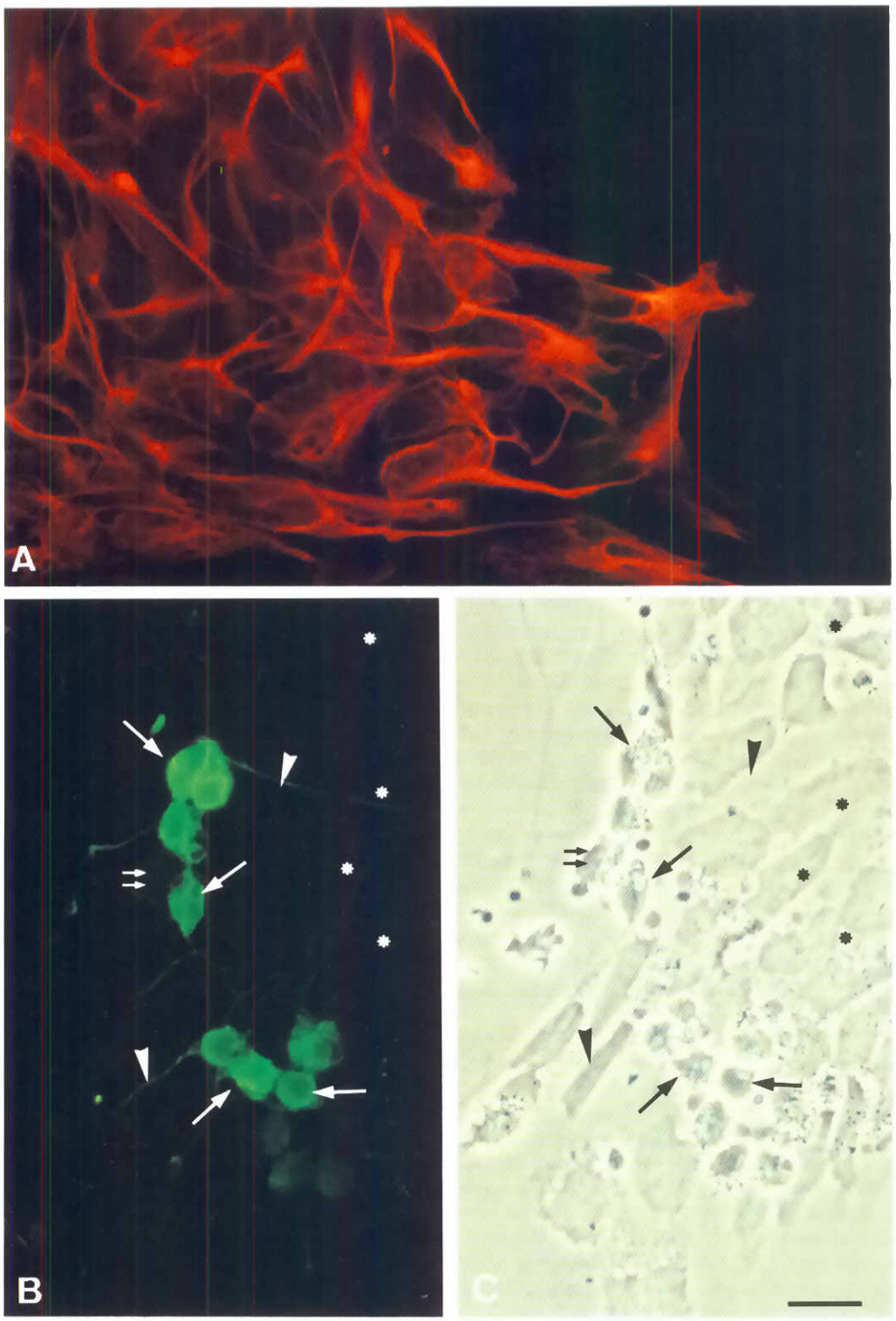

Fig. 3. Appearance of distinct cell lineages along neural induction without chordamesodermal influence (induction by dissociation of the target tissue). (A) *Differentiation of GFAp-positive cells presenting an astrocyte-like phenotype.* **(B, C)** *Differentiation of a GABAergic neuronal subpopulation. Cell bodies (arrows) as well as neurites (arrowheads) are labeled. Some neurons are negative (small arrows) indicating the appearance of different subsets in the neuronal population. Non-neuronal cells remain negative (asterisks). Bar= 20 μm.* ***(B)*** *immunofluorescence.* **(C)** *corresponding phase contrast.*

committed or were not able to express such a commitment when separated from this *in vivo* embryonic environment. This suggests that post-inductive events are mandatory for CAT and AChE expression in NF cells.

With respect to CAT expression, enhanced activity was observed only when neurectodermal cells were cultured intermingled with chordamesodermal cells. When the isolated cells from the two tissues (neurectoderm and chordamesoderm) were cultured sepa-

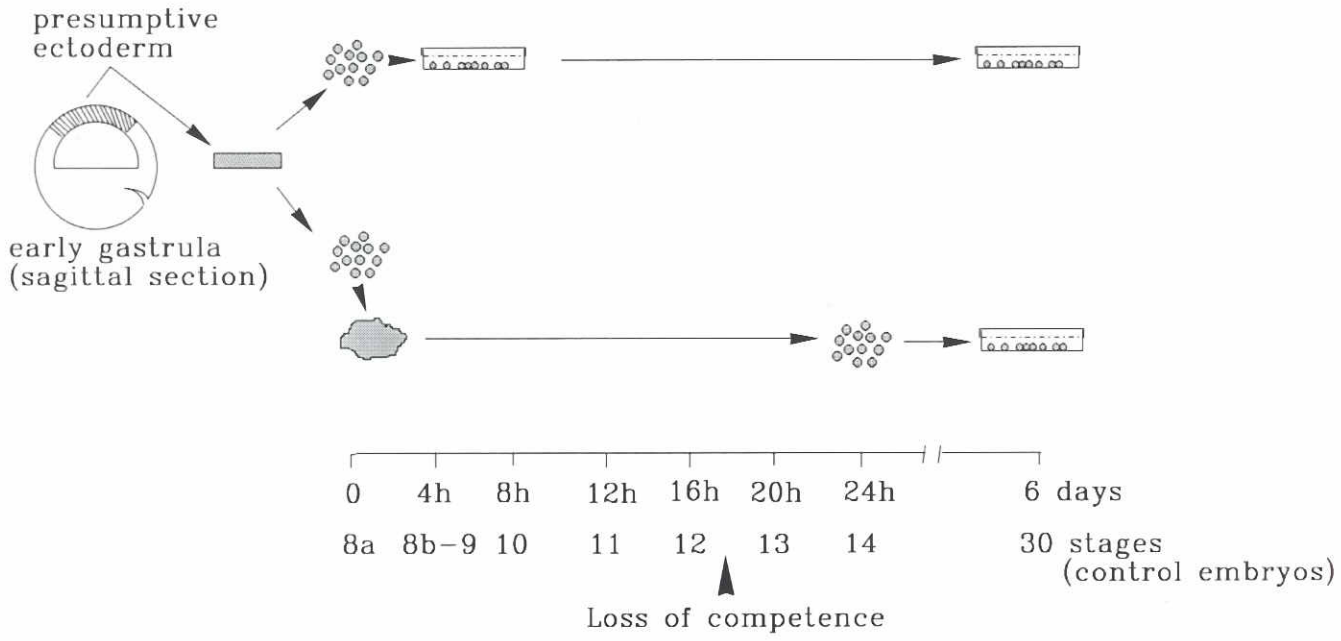

Fig. 4. Experimental procedure for *in vitro* induction by cell dissociation and cell reaggregation.

rately in the same dish or when the neural precursor cells were cultured in a medium previously conditioned on co-cultures, this effect was completely absent. In the same way, we found that extracellular matrix components failed to substitute for chorda-mesodermal cells in supporting the full expression of CAT activity (Huang *et al.*, 1990). These results suggest that optimal expression of cholinergic traits requires a further close contact between neurectodermal and chordamesodermal cells. This AChE pattern of expression was similar to that of the CAT. The A12 asymmetric form was only expressed in co-cultures.

This AChE isoform might relate to the formation of nerve-muscle junctions, since it was absent in isolated NP or/and NF and also in isolated CM cultures (Duprat *et al.*, 1990).

In NP cell cultures, the different globular type forms appeared, (G_4, G_2-G_1). In NF cell cultures, a very small peak of G_2-G_1 AChE isoforms was barely detectable. However in the latter cultures a positive specific staining was showed using the Karnovsky and Roots cytoenzymological method with iso-OMPA, a specific pseudo-cholinesterase inhibitor.

From recent data on peptidergic, adrenergic and gabaergic initial expressions in amphibian neurectoderm (Pituello *et al.*, 1989a, 1989b), we can now conclude that the stimulating effect observed when neuroblast cells were co-cultured with differentiating chorda-mesodermal cells was not restricted to cholinergic traits. These new experiments, carried out in order to better understand the mechanism of chordamesodermal influence, indicated that all enzymes quantitatively studied (tyrosine hydroxylase, dopa decarboxylase, dopamine beta-hydroxylase for catecholamines or glutamic acid decarboxylase for GABA) were present in NP and in NF cultures and were stimulated in co-cultures.

The molecular mediation of stimulation by chordamesoderm is under investigation.

The data summarized here demonstrate that as a consequence of neural induction, neural precursor cells acquire the ability to undergo a high degree of structural, neurochemical and functional differentiation. We recorded the initial appearance and expression of different neurotransmitter phenotypes. Biosynthesis, accumulation, uptake, release and degradation properties are expressed in isolated NP cell cultures as well as in isolated NF cell cultures, except, in the latter, for cholinergic phenotype. Our experiments also provide further evidence that the process of dissociating cells and growing them in culture does not substantially change their developmental program. By staging sibling embryos of ages equivalent to those of cells grown *in vitro* we have shown that differentiation of neuroblasts in culture follows a time course similar to that observed *in vivo* (Pituello *et al.*, 1989a, 1989b).

Emergence of different neural lineages without further chordamesodermal influence after neural induction

Using immunocytochemical visualization of specific markers for different neuronal phenotypes, we demonstrated the emergence of distinct subpopulations in NP and in NF cultures as well as in co-cultures (Pituello *et al.*, 1989a, 1989b). Thus, the expression of neuronal phenotypes (somatostatin, leu-enkephalin, met-enkephalin, substance P, GABA, catecholamines, etc.) in different subsets of the neuronal population differentiated in culture suggested that certain neuronal precursors become committed to different metabolic pathways at the earliest steps of neurogenesis. During neural induction some neuronal precursor cells acquired the ability to express the neurotransmitter or neuropeptide-related phenotypes (specific gene regulations) without requiring, subsequently, further cues from the chordamesoderm. These original data are of importance. They suggest that at the late gastrula stage the neural precursor population does not constitute a homogeneous set of cells.

It was now of interest to know the origin of this phenomenon. Is this neuronal heterogeneity a direct result of the process of neural

induction itself (stochastic phenomena being involved or not at the cellular level), or does it reflect a pre-existing heterogeneity in the presumptive ectoderm, target tissue of neural induction?

Distinct neural predispositions of presumptive ectodermal cells

The rapid appearance of diversity in the neural cell population isolated just after neural induction, dissociated and grown in a defined saline medium, raises questions concerning its origin and notably whether it reflects heterogeneity in the inductive tissue or in the target tissue or both.

It is now well evidenced that the inducing tissue itself seems to have no real specificity in neural commitment (cf. Nieuwkoop *et al.*, 1985; Holtfreter, 1988; Saxén, 1989). The temporally limited competence of the target tissue highlights its importance and suggests that it already has some inherent propensity for neuralization.

Last year, in agreement with pioneer observations by Holtfreter (1945), we showed that at the early gastrula stage disaggregation of the competent presumptive ectoderm into isolated cells is sufficient to commit some cells along the neural pathways (Saint-Jeannet *et al.*, 1989b).

This experimental artifice allowed us to study the capacities of presumptive ectodermal cells to express different neural phenotypes, in the total absence of interaction with the natural inducing tissue (chordamesoderm).

Using immunological probes we have demonstrated for the first time that the neural population differentiated under dissociating conditions is composed of the two distinct neural lineages, neurons and glial cells.

- Immunostaining on 6-day-old cultures revealed a large population of cells presenting reactivity to the antibody directed against GFAp (glial fibrillar acid protein) (Fig. 3).
- Neuronal markers, neurofilament polypeptides and tetanus toxin binding sites were also immunodetected in neurons. 5% of the total number of cultured cells displayed this neuronal phenotype. This number is equivalent to the number of neuronal cells obtained after *in vitro* induction involved by association (during 4 h) of the presumptive ectoderm with the natural inducing tissue.

In order to answer the question "do these neurons express different neurotransmitter-related phenotypes just as they do after induction *in vivo* during gastrulation?", presumptive ectodermal cells neuralized by dissociation and cultured up to 6 days were processed for biochemical assays and immunocytochemical detection of the main neuronal phenotypes.

Two identifiable subpopulations of neurons differentiated among neuronal cells without neurotransmitter characteristics (Fig. 3 B-C). Thus, we pointed out that the developmental neuronal repertoire of ectodermal cells induced without interaction with the chordamesoderm appears relatively restricted compared to that of "naturally" neuralized ectoderm (Saint-Jeannet *et al.*, submitted).

Taken together these data indicate that dissociation procedure of the competent target tissue results in the commitment of cells towards the neuronal and glial pathways. They are consistent with the notion that the competent presumptive ectoderm possesses at the early gastrula stage a limited number of developmental predispositions before neural induction. These can be materialized in the form of different neuronal and glial lineages and neuronal subpopulations as a consequence of a permissive inductive signal.

Early gastrula presumptive ectodermal cells constitute an apparently heterogeneous population presenting distinct predispositions to be neuralized with regard to neuronal and glial lineage segregation.

We demonstrated that the presumptive ectoderm undergoes neural induction in response to dissociation in a competence-dependent manner. The basic pH of the dissociation medium had no neural inducing effect on its own. A loss of cell-cell contacts (during the dissociation step) could be sufficient to elicit neural induction.

These data are consistent with the idea of an active role played by these *cell-cell contacts in the target tissue* for eliciting neural commitment *in vitro*. We also found that whereas rupture of cell-cell contacts evoked neural induction, dissociation immediately followed by cell-reaggregation reduces the neuralizing response (Fig. 4), indicating an active role played by target cell-cell contacts *in the modulation of cell commitment* (Gurdon, 1988; Saint-Jeannet *et al.*, 1989b). Cell community appeared to have a negative regulatory effect on the expression of the neural differentiation: more neurons were observed when the cells were in a monolayer sheet than when they were aggregated in three-dimensional structures. Induced dissociated cells, recovering their initial tissular structure during a reaggregation step, change their neural commitment towards an epidermal status. On the other hand, with regard to epidermal determination the community effect is a direct one (Saint-Jeannet *et al.*, submitted).

Taken together our results strongly suggest that early gastrula presumptive ectodermal cells might constitute a heterogeneous population, presenting distinct predispositions to be neuralized. Throughout induction without chordamesodermal cues (by dissociation of the target tissue) cells of the presumptive ectoderm can differentiate *in vitro* with the emergence of the two main neural lineages: neuronal lineage and glial lineage.

The question now concerns the molecular basis at the origin of the diverse predispositions of presumptive ectodermal cells. This could result in a different distribution of determinants along cleavage stages or could be a consequence of epigenetic events taking place just before gastrulation.

Acknowledgments

We gratefully acknowledge the expert technical assistance provided by F. Foulquier and M.J. Guinaudy. We are indebted to P. Cochardand M.C. Giess for suggestions and comments, and to C. Daguzan and C. Mont for help in preparation of this manuscript. Our work was supported by grants from the CNRS, the MRES and the EEC.

References

BARTH, L.G. and BARTH, L.J. (1959). Differentiation of cells of the *Rana pipiens* gastrula in unconditioned medium. *J. Embryol. Exp. Morphol. 7:* 210-222.

BORN, J., HOPPE, P., JANECZEK, J., TIEDEMANN, H. and TIEDEMANN, H. (1986). Covalent coupling of neuralizing factors from *Xenopus* to Sepharose beads: no decrease of inducing activity. *Cell Differ. 19:* 97-101.

BOUCAUT, J.C., DARRIBERE, T., BOULEKBACHE, H. and THIERY, J.P. (1984). Anti-fibronectin antibodies prevent gastrulation but do not perturb neurulation in gastrulated amphibian embryos. *Nature 307:* 364-367.

DUPOU, L., GUALANDRIS, L., LOPEZ, A., DUPRAT, A.M. and TOCANNE, J.F. (1987). Alterations in lateral lipid mobility in the plasma membrane of Urodelean ectodermal cells during gastrulation. *Exp. Cell Res. 169:* 502-513.

DUPRAT, A.M. and GUALANDRIS, L. (1984a). Extracellular matrix and neural determi-

nation during amphibian gastrulation. *Cell Differ. 14:* 105-112.

DUPRAT, A.M., GUALANDRIS, L., FOULQUIER, F., PAULIN, P. and BIZZINI, B. (1986). Neuronal induction and *in vitro* initial expression of neurofilament and tetanus toxin binding site molecule in amphibians. *Cell Differ. 18:* 57-64.

DUPRAT, A.M., GUALANDRIS, L., KAN, P. and FOULQUIER, F. (1984b). Early events in the neurogenesis of amphibians. In *The role of Cell Interactions in Early Neurogenesis* (Eds. A.M. Duprat, A. Kato , M. Weber). Plenum Press, New York, pp. 3-20.

DUPRAT, A.M., GUALANDRIS, L., KAN, P., PITUELLO, F., SAINT-JEANNET, J.P. and BOUDANNAOUI, S. (1987). Review: Neural induction. *Biol. Struct. Morphogen. 75:* 211-227.

DUPRAT, A.M., GUALANDRIS, L. and ROUGE, P. (1982). Neural induction and the structure of the target cell surface. *J. Embryol. Exp. Morphol. 70:* 171-187.

DUPRAT, A.M., KAN, P., FOULQUIER, F. and WEBER, M. (1985c). *In vitro* differentiation of neuronal precursor cells from amphibian late gastrulae: morphological immunocytochemical studies, biosynthesis, accumulation and uptake of neurotransmitters. *J. Embryol. Exp. Morphol. 86:* 71-87.

DUPRAT, A.M., KAN, P., GUALANDRIS, L., FOULQUIER, F., MARTY, J. (1985b). Neural induction: embryonic determination elicits full expression of specific neuronal traits. *J. Embryol. Exp. Morphol. 89:* 167-183.

DUPRAT, A.M., KAN, P. and WEBER, M. (1990). Initial expression of cholinergic traits in embryonic amphibian neuronal cells, choline acetyltransferase and acetylcholinesterase activities. *Ontogenez* (In press).

DUPRAT, A.M., ROUGE, P., FLOTTES, S. and BLEYS, M. (1985a). Lectin probes for visualization of cell differentiation markers on embryonic cells. In *Lectins, Vol. IV* (Eds. T.C. Bog-Hansen and J. Breborowicz). Walter de Gruyter & Co., New York, pp. 193-201.

DUPRAT, A.M., ZALTA, J.P. and BEETSCHEN, J.C. (1966). Action de l'actinomycine D sur la différenciation de divers types de cellules embryonnaires de l'amphibien *Pleurodeles waltl* en culture *in vitro*. *Exp. Cell Res. 43:* 358-366.

GUALANDRIS, L. and DUPRAT, A.M. (1987). Structural alteration of the target plasma membrane affects reception but not expression of the neural inductive signal. *Cell Differ. 20:* 147-152.

GUALANDRIS, L., ROUGE, P. and DUPRAT, A.M. (1983). Membrane changes in neural target cells studied with fluorescent lectin probes. *J. Embryol. Exp. Morphol. 77:* 183-200.

GUALANDRIS, L., ROUGE, P. and DUPRAT, A.M. (1985). Target cell surface glycoconjugates and neural induction in an amphibian. *J. Embryol. Exp. Morphol. 86:* 39-51.

GURDON, J.B. (1987). Embryonic induction. Molecular prospects. *Development 99:* 285-306.

GURDON, J.B. (1988). A community effect in animal development. *Nature 33:* 772-774.

HOLTFRETER, J. (1945). Neuralization and epidermization of gastrula ectoderm. *J. Exp. Zool. 98:* 161-209.

HOLTFRETER, J. (1988). A new look at Spemann's organizer. In *The Molecular Biology of Cell Determination and Cell Differentiation* (Ed. L.W. Browder). Plenum Press, New York, pp.127-150.

HUANG, S., SAINT-JEANNET, J.P., KAN, P. and DUPRAT, A.M. (1990). Extracellular matrix: an immunological and biochemical (CAT and TOH activity) survey of *in vitro* differentiation of isolated amphibian neuroblasts. *Cell Differ. Dev.* (In press).

NIEUWKOOP, P.D., JOHNEN, A.G. and ALBERS, B. (1985). *The epigenetic nature of early chordate development. Inductive interaction and competence.* Development and Cell Biology Series, 16. Cambridge University Press

OTTE, A.P., KOSTER, C.H., SNOEK, G.T. and DURSTON, A.J. (1988). Protein kinase C mediates neural induction in *Xenopus laevis*. *Nature 334:* 618-620.

OTTE, A.P., VAN RUN, P., HEIDEVELD, M., VAN DRIEL, R. and DURSTON A.J. (1989). Neural induction is mediated by cross-talk between the protein kinase C and cyclic AMP pathways. *Cell 58:* 641-648.

PITUELLO, F., DERUNTZ, P., PRADAYROL, L. and DUPRAT, A.M. (1989a). Peptidergic properties expressed *in vitro* by embryonic neuroblasts after neural induction. *Development 105:* 529-540.

PITUELLO, F., KAN, P., GEFFARD, M. and DUPRAT, A.M. (1989b). Initial gabaergic expression in embryonic neuroblasts after neural induction. *Int. J. Dev. Biol. 33:* 445-454.

SAINT-JEANNET, J.P., FOULQUIER, F., GORIDIS, C. and DUPRAT, A.M. (1989a). Expression of N-CAM precedes neural induction in *Pleurodeles waltl* (Urodele, Amphibian). *Development 106:* 675-683.

SAINT-JEANNET, J.P., HUANG, S. and DUPRAT, A.M. (1989b). Target cell contacts and neural commitment in *Pleurodeles waltl. Cell Differ. 27 (Supp.):* 165.

SAXÉN, L. (1989). Neural induction. *Int. J. Dev. Biol. 33:* 21-48.

SAXÉN, L. and TOIVONEN, S. (1962). *Primary Embryonic Induction.* Academic Press, London.

TIEDEMANN, H. and BORN, J. (1978). Biological activity of vegetalizing and neuralizing inducing factors after binding to BAC-cellulose and CNBr-Sepharose. *Roux Arch. Dev. Biol.184:* 285-299.