Differential Perturbations in the Morphogenesis of Anterior Structures Induced by Overexpression of Truncated XB- and N-Cadherins in *Xenopus* Embryos

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Abstract. Cadherins, a family of Ca-dependent adhesion molecules, have been proposed to act as regulators of morphogenetic processes and to be major effectors in the maintenance of tissue integrity. In this study, we have compared the effects of the expression of two truncated cadherins during early neurogenesis in *Xenopus laevis*. mRNA encoding deleted forms of XB- and N-cadherin lacking most of the extracellular domain were injected into the four animal dorsal blastomeres of 32-cell stage *Xenopus* embryos. These truncated cadherins altered the cohesion of cells derived from the injected blastomeres and induced morphogenetic defects in the anterior neural tissue to which they chiefly contributed. Truncated XB-cadherin was more efficient than N-cadherin in inducing these perturbations. Moreover, the coexpression of both truncated cadherins had additive perturbation effects on neural development. The two truncated cadherins can interact with the three known catenins, but with distinct affinities. These results suggest that the adhesive signal mediated by cadherins can be perturbed by overexpressing their cytoplasmic domains by competing with different affinity with catenins and/or a common anchor structure. Therefore, the correct regulation of cadherin function through the cytoplasmic domain appears to be a crucial step in the formation of the neural tissue.

THE development of multicellular organisms proceeds by successive waves of cell proliferation, cell migration, and cell condensation leading to extensive tissue rearrangements. The interactions of cells with their environment play crucial roles during these processes, and acquisition of selective adhesive properties are assumed to be required to achieve the correct development of embryonic tissues (Edelman, 1992; Takeichi, 1991). Cell–cell adhesion is mediated by several classes of molecules including those belonging to the immunoglobulin superfamily (Edelman et al., 1990; Springer, 1990) and the cadherin family (Geiger and Ayalon, 1992; Kemler et al., 1989; Takeichi, 1990). Cadherins are calcium-dependent cell surface glycoproteins with more than 15 members that share high sequence similarity (Koch et al., 1992; Magee and Buxton, 1991; Suzuki et al., 1991). The amino-terminal part of the extracellular domain of cadherins binds homotypically and links together adjacent cells expressing the same type of cadherin (Nose et al., 1990; Ozawa et al., 1991). The intracellular domain binds to catenins that may contribute to the anchorage of cadherins to the cytoskeletal network (Hirano et al., 1992; Kemler, 1993; Magee and Buxton, 1991; McCrea and Gumbiner, 1991; Nagafuchi et al., 1991; Ozawa et al., 1989, 1990*a*; Ozawa and Kemler, 1992; Schwartz et al., 1990; Troyanovsky et al., 1993). Mutations in the calcium-binding sites or deletions of part of the extracellular or cytoplasmic domain of cadherins result in a complete loss of adhesive function. In the latter case, the truncated cadherins are unable to bind to catenins and to anchor to the cytoskeleton (Ozawa et al., 1990*b*; Nagafuchi and Takeichi, 1988).

In vivo, the correlation between the spatiotemporal expression of some cadherins and morphogenetic events has been heavily documented in different species. For instance in chicken and mouse embryos, differential expression of N-, E- (L-cellular adhesion molecule [CAM][1], P-, R-, B-, M- and T-cadherins has been associated with cell migration, tissue segregation, and terminal differentiation during histogenesis (Donalies et al., 1991; Duband et al., 1987; Hatta

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1. *Abbreviations used in this paper*: CAM, cellular adhesion molecule; NAM, normal amphibian medium; VSVG, vesicular stomatitis virus glycoprotein; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside.

et al., 1987; Inuzuka et al., 1991; Moore and Walsh, 1993; Napolitano et al., 1991; Nose et al., 1987; Ranscht and Dours-Zimmermann, 1991; Sacristàn et al., 1993; Thiery et al., 1984). In vitro models have also provided some evidence that the modification of the repertoire of cadherins at the cell surface is strongly associated with changes in cell morphology and is capable of inducing cell sorting (Detrick et al., 1990; Edelman et al., 1987; Friedlander et al., 1989; Fujimori et al., 1990; Matsuzaki et al., 1990; Nose et al., 1988). Thus, a well-defined expression pattern of cadherins confers selective adhesive properties that may, in turn, act as a regulator of morphogenetic processes.

Recent studies have shown that a truncated form of cadherin, deleted in its extracellular or cytoplasmic domain, could act as a dominant-negative mutant (Fujimori and Takeichi, 1993; Kintner, 1992; Troyanovsky et al., 1993; Levine et al., 1994). This inhibition of the adhesive function of cadherins might involve a competition between the cytoplasmic domains of the deleted cadherins and the endogenous ones, either for the binding of the catenins or other cytoplasmic components (Kintner, 1992) or for the occupancy of the natural sites of expression of cadherins (Fujimori and Takeichi, 1993). A truncated form of E-cadherin deleted in the cytoplasmic domain can induce a selective disruption of cadherin function (Levine et al., 1994).

In *Xenopus laevis* embryos, one of the earliest cadherins detected is the maternally derived EP-cadherin, which is expressed at the cell surface during cleavage stages (Ginsberg et al., 1991; Levi et al., 1991*a*). XB-cadherin, another closely related cadherin and U-cadherin (Angres et al., 1991; Herzberg et al., 1991) were also reported to display a similar initial expression pattern. When tissue segregation begins to take place at the end of gastrulation, E-cadherin becomes expressed in the ectoderm (Choi and Gumbiner, 1989; Levi et al., 1991*b*), and N-cadherin appears in the neuroepithelium and the axial mesoderm (Detrick et al., 1990; Simonneau et al., 1992).

In the present study, we have analyzed the role of the XB- and N-cadherins during *Xenopus laevis* neurogenesis. We have generated truncated constructs for these cadherins, deleted in their extracellular domain, which can act as dominant-negative mutants. To target their expression to the prospective anterior neural territory, we have injected the corresponding mRNA into the four animal dorsal blastomeres of 32-cell stage embryos. The perturbation of both cadherins, which are expressed either before (XB-cadherin) or as a result of neural induction (N-cadherin), should give us some clues about the function of these molecules in the events that precede and follow neural tissue specification. We found that the expression of similar amounts of the truncated XB- or N-cadherin gives rise to different grades of developmental defects in anterior neural structures. When both mRNAs were coinjected, additive effects were clearly observed. These distinct perturbation effects on embryonic development resulting from the alteration of endogenous cadherin-mediated cell adhesion could reflect the different affinity properties of cadherin cytoplasmic domain for catenins. Therefore, in addition to the cadherin-mediated selective homophilic interaction expressed during embryonic development, the affinity of specific cadherin cytoplasmic domain for intracellular ligands should also regulate cell adhesion. These results highlight the relative importance of specific cadherin-mediated cell adhesion in the early and late events that control neural tissue formation in *Xenopus laevis*.

Materials and Methods

Animals

Adult *Xenopus laevis* were obtained from the "Service d'élevage de *Xénope*" of the Centre National de la Recherche Scientifique (Montpellier, France). Mature oocytes were stripped from females injected 12 h earlier with 1,000 IU of chorionic gonadotropin (Sigma Immunochemicals, St. Louis, MO) and immediately fertilized with a minced testis. Embryos were reared in normal amphibian medium (NAM) 0.1× (Slack, 1985) and staged according to Nieuwkoop and Faber (1967).

Generation of Truncated XB-Cadherin, N-Cadherin and N-CAM

All the constructs used for in vitro transcription were inserted into pSP35T (Amaya et al., 1991), a modified version of the pSP64T vector containing the 5′ and 3′ untranslated sequences of *Xenopus* β-globin gene (Krieg and Melton, 1984).

We have previously isolated two full-length cDNA that represent the two allelic forms of *Xenopus* N-cadherin, called pBS8Ncad and pBS9Ncad, respectively (Simonneau et al., 1992). The deleted N-cadherin construct was generated from pBS8Ncad. This modified cDNA codes for a N-cadherin deleted in its extracellular region from amino acid (aa) 31 to aa 699 (aa numbers refer to the immature protein).

In a first step, pBS8Ncad was digested by BspMI and SmaI, the cohesive ends generated by BspMI were filled, and all termini were dephosphorylated. The 4.2-kb fragment containing the coding sequence from aa 699 to the carboxy terminus of the N-cadherin, 810 bp of 3′ untranslated sequence and vector sequence, was purified from an agarose gel. In a second step, pBS8Ncad was digested by PvuII-AvaI, the cohesive ends were filled, and the 97-bp fragment was purified from an agarose gel. This fragment contains 12 bp of 5′ untranslated sequence, the cDNA region coding for the signal peptide, and 4 aa of the propeptide of N-cadherin. Both fragments were subsequently ligated, generating the N-cadherin–deleted cDNA construct called p8ΔNcad, which codes for the truncated N-cadherin. NcoI and SalI sites were created before the start codon and after the stop codon of the truncated cadherin cDNA by a PCR using specific oligonucleotides. The PCR insert was primed using an oligonucleotide carrying an NcoI restriction site at the 5′ end of the aminoterminal MGRKE aa sequence of the *Xenopus* N-cadherin (5′-TGCACCATGGGCCGAAAGAGC-3′) and an antisense oligonucleotide containing a SalI site at the 3′ end of the *Xenopus* N-cadherin carboxyterminal YGGSDD aa sequence (5′-CACTGTCGACTCAGTCGTCGCTCCCTCCGT-3′). The NcoI-SalI amplified fragment (912 bp) was subsequently inserted into the NcoI-SalI sites of the transcription vector pSP35T (a generous gift from Dr. M. Kirschner, Department of Biochemistry and Biophysics, University of California, San Francisco, CA). This construct called pΔNcad was subsequently used as a matrix to generate other deleted constructs.

A hybrid cDNA was generated where the cytoplasmic coding sequences of *Xenopus* N-cadherin were substituted for those of *Xenopus* XB-cadherin (Herzberg et al., 1991). Synthetic oligonucleotides were made, the first coding for the *Xenopus* XB-cadherin cytoplasmic IVVKEPL aa sequence and containing an in-frame SspI site at its 5′ end (5′-GCGACCAATATTGTTGTGAAAGAGCCACTA-3′). The second is the complementary nucleotide sequence of the carboxy terminal DDDDEE aa sequence of *Xenopus* XB-cadherin with a SalI site at its 3′ end (5′-ACGCCACGTCGACTACTCTTCATCATCATCA-3′). Both oligonucleotides were used for a PCR amplification of the XB-cadherin cDNA. The resulting SspI-SalI–digested primed insert (471 bp) was exchanged with the SspI-SalI insert of pΔNcad, generating a hybrid-deleted cDNA. This construct, called pΔNXBcad, codes for *Xenopus*-deleted N-cadherin aa sequence from aa 1 to aa 30 and aa 699 to aa 769, linked to the XB-cadherin cytoplasmic aa sequence between aa 303 and aa 446 (aa numbers are according to the XB-cadherin sequence published by Herzberg et al., 1991).

Another construct was generated that codes for an N-cadherin/N-CAM hybrid protein. The N-CAM sequence coding for the transmembrane and cytoplasmic domains of the 140-kD mol mass spliced form were juxtaposed to the 41 amino-terminal aa of the pΔNcad sequence by the following procedure: the DNA fragment of the *Xenopus* N-CAM 140 sequence, encoding

the last six aa of the extracellular domain to the carboxyterminus and lacking the exon 18 sequence, was produced by PCR amplification using specific oligonucleotides of a *Xenopus* liver cDNA library prepared from adult *Xenopus* liver. Briefly, liver RNAs were prepared from adult *Xenopus laevis* using the guanidinium-phenol-chloroform method (Chomczynski and Sacchi, 1987) and cDNA synthesis was carried out using a cDNA synthesis kit (Boehringer Mannheim Biochemicals, Indianapolis, IN) from 2 μg of poly A^+ mRNAs prepared with the mRNA preparation kit (Pharmacia Fine Chemicals, Piscataway, NJ). The N-CAM 140 fragment obtained by PCR was primed at the 5′ end with an oligonucleotide containing a PstI site in frame with the GLGTGA aa sequence of the *Xenopus* N-CAM (5′-CGGATCCTGCAGCGGTTTGGGAACAGGAGCA-3′), and at the 3′ end with an oligonucleotide containing a SalI site and the NESKA aa sequence of the *Xenopus* N-CAM (5′-CTTGAGCGTCGACTCATGCTTTGCTCTCATT-3′). These oligonucleotide sequences were chosen according to the *Xenopus* N-CAM coding sequence previously published (Krieg et al., 1989). This insert (558 bp) was digested with both restriction enzymes and subsequently introduced into the SalI and XbaI sites of pΔNcad (the SalI site was 121 pb far from the start codon and the XbaI site was located just after the stop codon of pΔNcad). This construct was called pΔNcadNCAM.

All the constructs were subsequently checked by sequence analysis using the Sequenase™ kit (Amersham Corp., Arlington Heights, IL).

Introduction of an Epitope "Tag" into the Truncated Constructs

To detect the truncated proteins expressed after injection into blastomeres, we introduced into the coding sequences of the truncated proteins, a "tag" sequence coding for the YTDIEMNRLGK aa sequence of the carboxyterminal tail of the vesicular stomatitis virus glycoprotein (VSVG) that can be specifically recognized by monoclonal antibody P5D4 (Kreis, 1986). This epitope, designated VSVG-11, has been juxtaposed to a hinge sequence coding for GPPGP at its amino-terminal extremity to improve its accessibility in the protein; the resulting GPPGPYTDIEMNRLGK aa sequence is called VSVG-16. The KS-pCV6-Nter-VSVG plasmid (a generous gift from Dr. B. Goud, Institut Pasteur, Paris, France) was used as a matrix to amplify the VSVG-16 coding sequence by PCR and to create an in-frame PstI restriction site at its 5′ and 3′ ends. The insert was primed with an oligonucleotide coding for GPPGPY aa sequence of VSVG-16 epitope with an in-frame PstI site at its 5′ end (5′-CATACCTGCAGTGGCCCACCAGGCCCATAC-3′) and an oligonucleotide coding for the complementary MNRLGK aa sequence of the VSVG-16 epitope with an in-frame PstI site at its 3′ end (5′-TTAGCGTCGACTTGCCCAGCCGGTTCATCT-3′). This insert (73 bp) was subsequently digested with PstI restriction enzyme and introduced into the PstI site into the extracellular coding sequence of the truncated cadherin coding sequence. The constructs were subsequently checked by sequence analysis. The VSVG-16 epitope insertion into the constructs is indicated in Fig. 1, *A–C*.

In Vitro Transcription of the Truncated Constructs

For in vitro transcription, all constructs were linearized at the end of the 3′-untranslated *Xenopus* β-globin sequence and transcribed using SP6 polymerase according to Melton et al. (1984). Control mRNA was obtained from pSP64T vector coding for β-galactosidase (a generous gift from Dr. C. Kintner, Salk Institute for Biological Sciences, San Diego, CA; Detrick et al., 1990). mRNA transcribed in vitro by SP6 polymerase were checked with a rabbit reticulocyte lysate translation assay (Amersham Corp.).

Embryo Injections

Embryos were dejellied at the two-cell stage in 2% cysteine (pH 7.8) and immediately transferred at 16°C into NAM 0.1× plus 5% Ficoll containing penicillin and streptomycin until they reached the 32-cell stage. The four animal dorsal blastomeres were injected with 2–5 nl of mRNA using an Eppendorf pressure-driven microinjector (i.e., 0.025–1.25 ng of mRNA per blastomere). Dorsal blastomeres were identified according to the criteria of pigmentation and size (Fig. 3 *C*) described by Nieuwkoop and Faber (1967). After injection, the embryos were maintained in the NAM/Ficoll solution for 15 h at 23°C and were thereafter transferred into NAM 0.1× solution for further development.

In Vivo Translational Efficiency of Truncated mRNA

12 *Xenopus* embryos at the two-cell stage were injected with pΔNXBcad, pΔNcad, or pΔNcadNCAM mRNA (6.25 ng) and 0.3 μCi of ^{35}S-Translabel (ICN Biomedical, Inc., Costa Mesa, CA). Each batch of 12 embryos reaching stage 13NF were homogenized in 600 μl of cooled immunoprecipitation buffer (100 mM NaCl, 10 mM Tris, pH 7.5, 2 mM $CaCl_2$, 1% Triton X-100, 1% NP-40, 0.1% SDS, and protease inhibitor cocktail); the homogenate was centrifuged for 10 min at 10,000 rpm in a microfuge to clear the extract of yolk and lipids. The clarified supernatants were incubated under agitation with the P5D4 monoclonal antibody for 2 h at 4°C. The extracts were then incubated with 100 μl of protein G–plus-agarose beads (Oncogene Sciences, Paris, France) for 1 h, and the beads were subsequently separated by low speed centrifugation. After five washes in the immunoprecipitation buffer, the beads were boiled for 10 min in 100 μl of Laemmli sample buffer and were then removed by centrifugation. The immunoprecipitated proteins were fractionated on a 12% SDS-polyacrylamide gel. The gel was subsequently fixed, incubated with amplify solution (Amersham Corp.), dried, and autoradiographed. The autoradiography was digitalized using a video camera (612F; Data Copy) linked to an acquisition module and a graphic control unit that was designed by the staff of the Computer Science Laboratory of the Ecole Normale Supérieure. A band profile was obtained for each truncated protein made in the translational assay. These profiles were subsequently analyzed using the HERMeS software (Vincent et al., 1986) to quantify the intensity of each band (arbitrary units).

Coprecipitation of Catenins by Truncated Cadherins

12 *Xenopus* embryos at the two-cell stage were injected with pΔNXBcad or pΔNcad mRNA and ^{35}S-Trans-label as previously described. Each batch of 12 embryos reaching stage 13NF were homogenized in 600 μl of SDS-free immunoprecipitation buffer (100 mM $NaPO_4$, pH 7.2, 2 mM $CaCl_2$, 1% Triton X-100, 1% NP-40, and protease inhibitor cocktail). After carrying the same immunoprecipitation procedure that was used to check mRNA translation efficiency, the catenins that coprecipitated with the truncated cadherins were identified on a 7.5% SDS-polyacrylamide gel. The autoradiography was scanned as previously described.

Detection of β-Galactosidase Activity

Embryos were processed for X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) staining as described in Detrick et al. (1990). Briefly, the embryos were fixed in 2% paraformaldehyde (PFA) and 2% glutaraldehyde in PBS for 1 h on ice. After two rinses in PBS, embryos were incubated in 10 mM $K_4Fe(CN)_6$, 10 mM $K_3Fe(CN)_6$, 1 mM $MgCl_2$, 0.1% Triton X-100, 0.15% X-gal (Sigma Immunochemicals) in PBS overnight at 37°C. Subsequently, the embryos were dehydrated, depigmented for 2 h in 70% methanol, 10% H_2O_2, and clarified in benzyl alcohol/benzyl benzoate (1 vol/2 vol) for 15 min. The embryos were photographed and then transferred twice for 15 min in xylene, embedded in paraplast, and sectioned as described by Levi et al. (1987).

Antibodies

A rabbit polyclonal antibody made against *Xenopus* N-CAM was kindly provided by Dr. Gerald Edelman (Scripps Clinic, La Jolla, CA; Levi et al., 1990). Monoclonal antibodies 12/101 specific for skeletal muscle (Kintner and Brockes, 1985) and 4D specific for N-CAM 180 kD (Watanabe et al., 1986) were purchased from the Developmental Studies Hybridoma Bank maintained by the Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine (Baltimore, MD), and the Department of Biological Sciences, University of Iowa (Iowa City, IA), under contract N01-HD-6-2915 from the National Institute of Child Health and Human Development. The P5D4 monoclonal antibody, directed against the carboxy terminus of the vesicular stomatitis virus glycoprotein (Kreis, 1986), was a gift from Dr. B. Goud and was used to detect the "tag" sequence of the truncated proteins. The anti-rabbit-Ig antibody conjugated to fluorescein or peroxidase and the anti-mouse-Ig antibody conjugated to rhodamine or peroxidase were purchased from Amersham Corp.

Immunodetection

Embryos were fixed in isopentane, cooled in liquid nitrogen as described in Levi et al. (1987), embedded in paraplast, and 7.5-μm sections were prepared. The immunohistochemistry was performed as described by Saint-Jeannet et al. (1992). For whole-mount immunocytochemistry, vitelline membrane-free embryos were fixed for 2 h in 20% DMSO/80% methanol, transferred into 10% (final concentration) hydrogen peroxide diluted in the fixative, and incubated at room temperature for 2 d. After bleaching, the

embryos were treated exactly as described in Dent et al. (1989). The skeletal muscle antigens expressed by the embryos were revealed using the 12/101 monoclonal antibody followed by an incubation with an anti-mouse Ig conjugated to the horseradish peroxidase. After detection of the peroxidase activity, the embryos were observed and subsequently treated with the 4D antibody specific for *Xenopus* N-CAM followed by horseradish-peroxidase-coupled anti-mouse Ig.

Histological Staining

The embryos were fixed for 1 h in PFA 2% plus glutaraldehyde 2% in PBS. After two rinses in PBS and one in water, the embryos were dehydrated and embedded in paraplast as described in Levi et al. (1987). 5-μm paraplast sections were collected on gelatin-coated glass slides, deparaffinized in xylene, and rehydrated. Sections were then stained using the Ramon y Càjàl trichrome according to Gabe (1968). Briefly, sections were incubated 10 min in Zielh's fuschin (1/10; vol/vol), rinsed for 5 min in water, then washed for 1 min in 0.4% acetic acid before 10 min incubation in 0.4% indigocarmin prepared in picric acid-saturated water. After 2 min dehydration in 100% ethanol, the sections were transferred to xylene and mounted in Eukitt mounting medium (Prolabo, Fontenay-sous-Bois, France).

Quantification of Cell-Cell Disruption

To determine the sequence of events that lead to cell dissociation after injection of truncated cadherin mRNA, the embryos were micro-cinematographed using a time-lapse video apparatus (Mitsubishi video tape recorder 6720; Mitsubishi Kasei Corp., Tokyo) connected to a binocular (Nikon Inc., Melville, NY) through a Panasonic color camera (WV-CD130L; Matsushita Electric Industrial Co., Ltd., Osaka, Japan). Videos were then analyzed on a Panasonic color monitor (M1400; Matsushita Electric Co., Ltd.). Cell-cell disruption was quantified at the neurula stage (stage 15-17) by estimating the number of cells expelled from the embryos. A low number of cells released (ln) referred to an approximate loss of several tens of cells; a high number of cells released (hn) referred to several hundreds of cells released from the embryos.

Quantification of Retinal Mass in Control and Injected Embryos

Serial sections of control or injected embryos showing either perturbations in their retinal layers or fused retina were observed at a magnification of 200 using a video camera fixed on a binocular system (SMZ-U zoom; Nikon Inc., Melville, NY) and connected to a TV monitor. Retinal areas observed on each section were manually plotted on the TV monitor screen and copied onto a transparent paper. The transparent paper area drawings that exactly corresponded to the retinal areas for each embryo analyzed were weighed, thus giving arbitrary units to determine the retinal mass values.

Results

pΔNcad, pΔNXBcad, and pΔNcadNCAM cDNA Constructs and In Vivo Assay for the mRNA Translational Efficiency

A portion of the extracellular domain of the *Xenopus* N-cadherin pBS8Ncad sequence (Simonneau et al., 1992) was deleted to generate a cDNA coding for an N-cadherin truncated between aa 31 in the propeptide sequence and aa 699

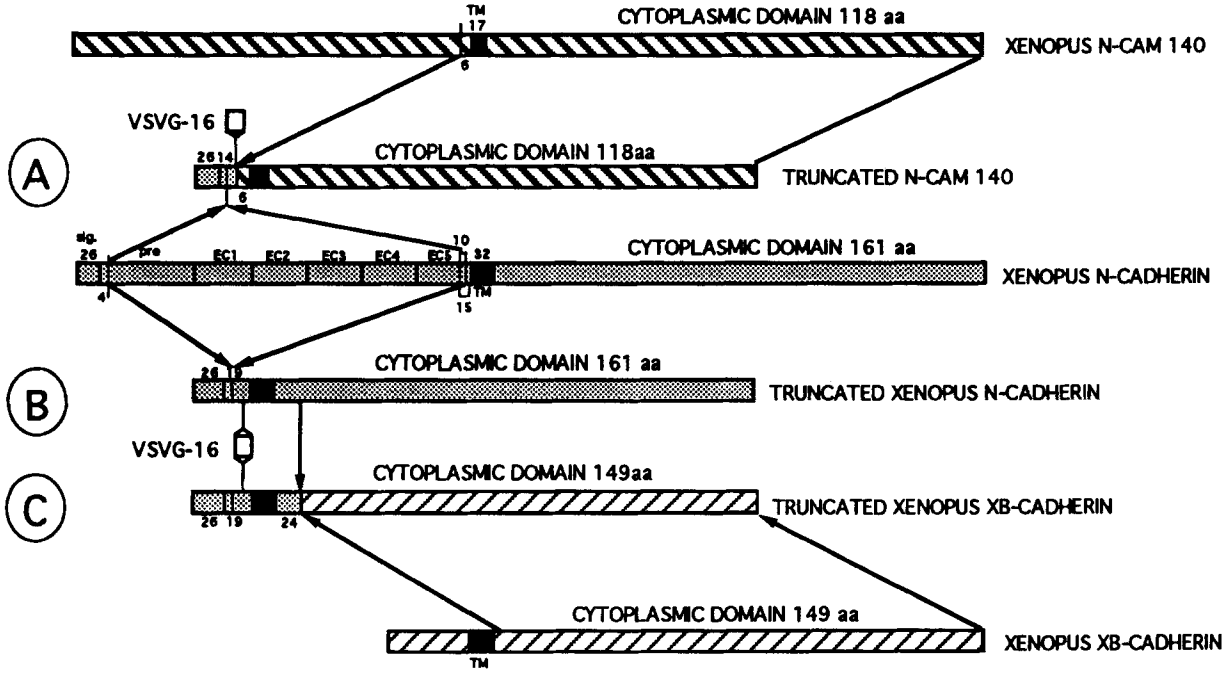


Figure 1. Schematic representation of the truncated NXB-, and N-cadherin, and NcadNCAM hybrid. (*A*) The truncated *Xenopus* N-cadherin/N-CAM hybrid protein is encoded by the pΔNcadNCAM mRNA and contains N-CAM transmembrane and cytoplasmic domains of the 140-kD mol mass spliced variant form juxtaposed to the 40 aminoterminal aa of the pΔNcad sequence (*B*) The truncated *Xenopus* N-cadherin protein is deleted in its extracellular region from aa 31 to aa 698, and it is encoded by the pΔNcad mRNA. (*C*) The truncated *Xenopus* N-cadherin/XB-cadherin hybrid protein contains N-cadherin aa sequences from aa 1 to aa 30 and aa 699 to aa 769 linked to the XB-cadherin aa sequences between aa 303 and aa 446. The tag VSVG-16 epitope is localized between aa 40 and 41 of the extracellular domain of all the truncated constructs. *TM*, transmembrane domain; *EC*, extracellular domain; *sig*, signal peptide; *pre*, propeptide sequence; VSVG-16, vesicular stomatitis virus glycoprotein carboxyterminal epitope recognized by a specific P5D4 monoclonal antibody and used as a tag for immunocytochemistry and whole-mount immunostaining.

in the EC5 domain. The VSVG-16 "tag" epitope was inserted between aa number 40 and 41 of the extracellular truncated domain. The transmembrane and cytoplasmic sequences were unchanged. This pΔNcad construct codes for the truncated N-cadherin and contains 253 aa (Fig. 1 *B*). This construct was then used to generate deleted constructs for XB-cadherin and N-CAM. An N-cadherin/XB-cadherin hybrid cDNA was obtained by exchanging the cytoplasmic coding sequence of N-cadherin with that of XB-cadherin. Both constructs contained the truncated extracellular domain with the tag epitope, the transmembrane domain, and the first 24 aa of the cytoplasmic domain of N-cadherin. The pΔNXBcad construct codes for an N-cadherin/XB-cadherin hybrid protein of 266 aa (Fig. 1 *C*).

A control construct was generated that codes for a truncated N-cadherin/N-CAM hybrid protein containing 197 aa (Fig. 1 *A*; pΔNcadNCAM). It contained the 40 amino-terminal aa of the truncated N-cadherin juxtaposed to the tag epitope and the *Xenopus* N-CAM sequences from the last 6 aa of the extracellular domain to the carboxy terminus. This hybrid codes for the 140-kD spliced cytoplasmic domain of *Xenopus* N-CAM.

All these deleted constructs were linearized at the end of the 3′ untranslated *Xenopus* β-globin sequence and transcribed using SP6 polymerase as described by Melton et al. (1984).

The translational efficiency of each mRNA was tested in vivo by coinjecting them with ^{35}S-Trans-label in two-cell stage embryos. Extracts prepared from stage 13NF embryos were subjected to immunoprecipitation with anti-tag monoclonal antibody. Fig. 2 shows the immunoprecipitated proteins analyzed on 12% SDS-polyacrylamide gels. The truncated NCadNCAM hybrid has an apparent molecular mass of 30 kD (lane *a*), two bands of 21 and 22.1 kD are obtained for truncated N-cadherin (lane *b*), two bands of 24.5 and 27.4 kD are also observed in the case of truncated pΔNXBcad mRNA injection (lane *c*). The upper band observed in lanes *b* and *c* could correspond to a form of truncated cadherins still carrying the signal peptide. The autoradiography was subsequently scanned and the intensity calculated (arbitrary units) for each band. An intensity of 25 was found for the truncated NCadNCAM hybrid. The intensity was, respectively, 133 and 51 for the lower and upper band corresponding to the truncated N-cadherin, while it was, respectively, 83 and 65 for the truncated NXB-cadherin. Taking into account the fact that the truncated NCadNCAM hybrid contained one methionine instead of five for the truncated cadherins, these results indicate that all the mRNA constructs have a similar in vivo translational efficiency.

Fate Map of Injected Blastomeres

The four animal-dorsal blastomeres (4-AD) of 32-cell stage embryos, referred to as blastomeres A1 A2 and their A'1 A'2 counterparts across the midline (Dale and Slack, 1987) have been shown in several fate map studies to contribute mainly to the anterior neural structures of the embryo (Moody, 1987; Dale and Slack, 1987; Jacobson and Hirose, 1981). Therefore, to target the presumptive neural blastomeres, the 4-AD blastomeres were chosen as the site of injection (Fig. 3 *C*, *arrowheads*).

In a first set of experiments, the 4-AD blastomeres were injected with mRNA encoding β-galactosidase to test the injection procedure and check for the progeny of the injected blastomeres.

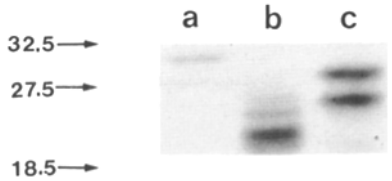


Figure 2. In vivo translation ability of mRNA coding for truncated cadherins or truncated N-cadherin/N-CAM hybrid. The mRNA were coinjected with ^{35}S-trans-label mixture at the two-cell stage of *Xenopus* embryos. At stage 13-13.5NF, the embryos were homogenized in immunoprecipitation buffer and incubated with anti-VSVG monoclonal antibody P5D4. The radiolabeled proteins immunoprecipitated were loaded on 12% acrylamide gel and revealed by autoradiography. Lane *a*, truncated NcadNCAM hybrid; lane *b*, truncated N-cadherin; lane *c*, truncated NXB-cadherin. Low molecular mass markers electrophoretic mobility are indicated on the left.

At stages 23-26, the injected embryos were fixed, processed for an X-gal staining, and photographed. As expected, the anterior structures such as the cement gland, the optic cups, and the proencephalon expressed strong β-galactosidase activity without any apparent defect in their organization (Fig. 3, *A* and *B*). The diencephalon, the anterior ectoderm, and the head mesenchyme were stained to a lesser extent. Occasionally, a faint staining could also be observed in the notochord and in few somitic cells. The embryos were subsequently embedded in paraffin and sectioned. Labeled β-galactoside–positive cells were found in well-organized neuroepithelia of the eye cup and the brain, as well as in the cement gland (Fig. 3 *D*). These results are consistent with the previously published fate maps for the 32-cell stage of *Xenopus laevis*.

Injection of mRNA Encoding the Truncated Cadherins Induce Developmental Defects

Embryos injected at the 32-cell stage were examined at stage 32NF. They were fixed and analyzed by a sequential whole-mount immunostaining with anti-skeletal muscle monoclonal antibody and then with anti-N-CAM monoclonal antibody. The results of each immunostaining were observed separately and the double whole-mount immunostaining is shown on Fig. 4, *A–E*. Embryos injected with pΔNXBcad mRNA exhibit extended malformations of the anterior neural structures as revealed with the anti-N-CAM antibody. The emergence of cranial nerves is delayed, the development of the eye is abnormal and delayed and numerous N-CAM–positive cell clusters are observed on each side of the mesencephalon (Fig. 4, *arrows* in *A* and *arrowheads* in *B*) in contrast to control embryos (Fig. 4, *D*, *arrows*, and *E*). The posterior structures and somites did not appear to be affected as revealed with the 12/101 antibody. At stage 41NF, the morphological appearance of injected embryos showed significant defects in eye development and in structures such as the cement gland and the anterior nervous system (Fig. 4 *C*, *arrows*) as compared to the control embryo (Fig. 4 *F*). Whole-mount immunostaining was also performed on injected embryos at stage 43NF with 4D and 12/101 monoclonal anti-

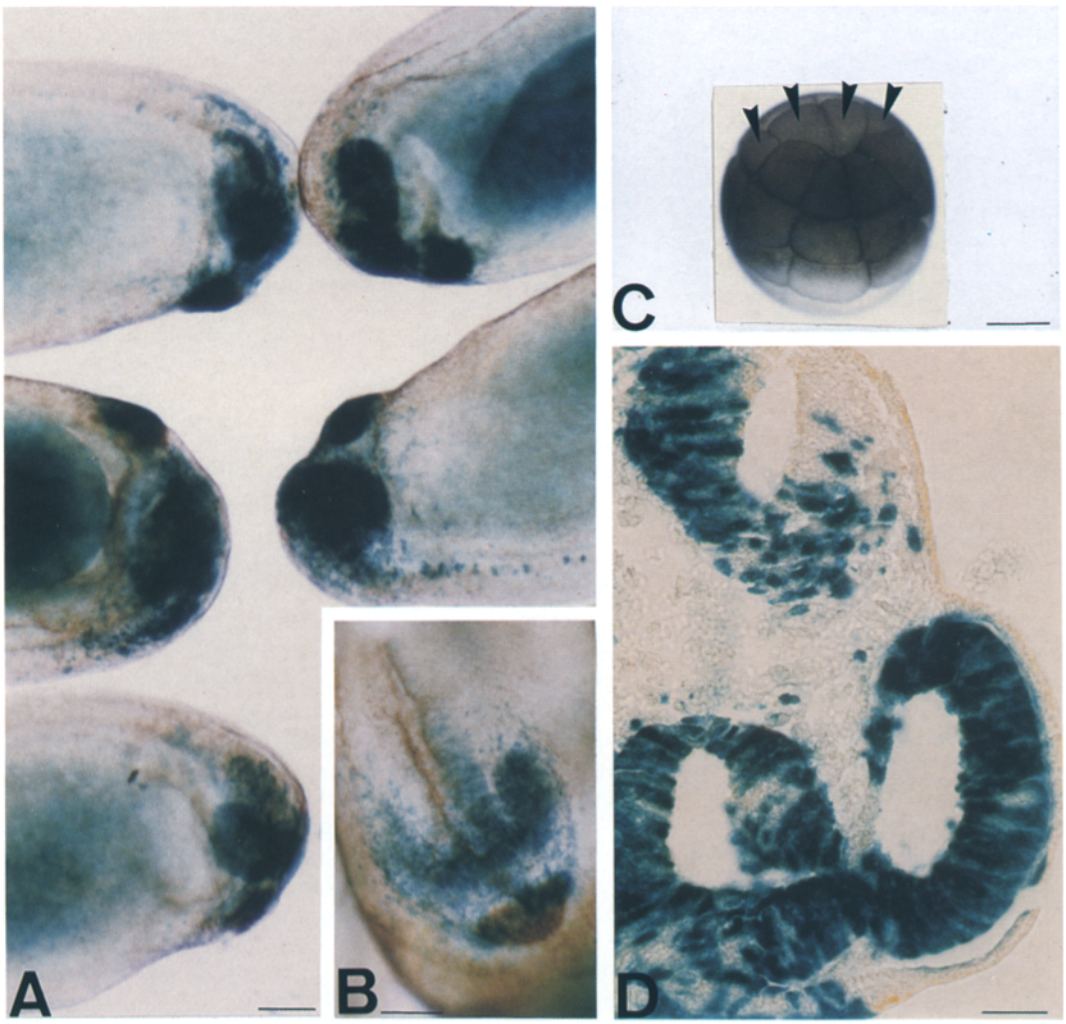


Figure 3. Fate map of the four animal dorsal blastomeres injected at the 32-cell stage. (*A*) Lateral view of embryos injected with 0.25 ng of β-galactosidase mRNA (1× concentration) at the 32-cell stage and processed for X-gal staining at stage 25NF. X-gal activity is restricted to the anterior neural structures. Staining in the endodermal mass is artefactual. (*B*) In the inset, a dorsal anterior view of an injected embryo where X-gal staining is localized to the optic vesicles, the brain, the cement gland, and to a lesser extent in the ectoderm and head mesenchyme. (*C*) Injection sites on a 32-cell stage Xenopus embryo. The four animal dorsal blastomeres (4-AD) A1, A2 (Dale and Slack, 1987) and their A'1, A'2 counterparts across the midline (*arrowheads*) were the target blastomeres. They are less pigmented and smaller in size than blastomeres of the ventral side. (*D*) Embryos injected with β-galactosidase mRNAs are processed for X-gal staining before sectioning at stage 25NF. Cells expressing the β-galactosidase activity are restricted to the neural lineage and the cement gland. Bar, 260 μm in *A*, 200 μm in *B*, 400 μm in *C*, and 106 μm in *D*.

bodies. The embryos injected with pΔNXBcad mRNA possessed numerous differentiated neuronal aggregates or single neurons with long processes localized under the ectoderm. An abnormal and extensive fasciculation of nerves emerging from the cranial ganglia was found. Some defects in the head skeletal muscles and hyperplasia of neural structures were also observed (data not shown).

Quantification of the perturbations obtained after mRNA injections was performed at stage 41NF. Increased concentrations of injected pΔNXBcad and pΔNcad mRNAs resulted in an increase in the percentage of embryos with aberrant morphology (Fig. 5 *A*). At a low concentration (0.1×), pΔNXBcad mRNA induced more malformations than pΔNcad mRNA: 17.7% of the embryos had developmental defects ($n = 17$) in the case of the pΔNXBcad mRNA injections, while no malformations were detected in the case of pΔNcad mRNA ($n = 13$) or pΔNcadNCAM mRNA ($n = 12$) injections. An increase in the amount of pΔNcad mRNA injected induced developmental defects in 20% ($n = 30$) and 34.8% ($n = 23$) of the cases examined for the 1× and 5× concentrations, respectively, whereas pΔNXBcad mRNA at 1× and 5× concentrations resulted in high numbers of embryos with malformations: 63% ($n = 38$) and 72.3% ($n = 18$), respectively. Injection of control pΔNcadNCAM mRNA at 1× and 5× concentrations induced malformations at only low frequency in 9% ($n = 12$) and 16% ($n = 25$), respectively, of injected embryos.

To analyze this differential effect on developmental abnormalities produced by pΔNXBcad or pΔNcad mRNA injections, we performed coinjection of both mRNA. Each

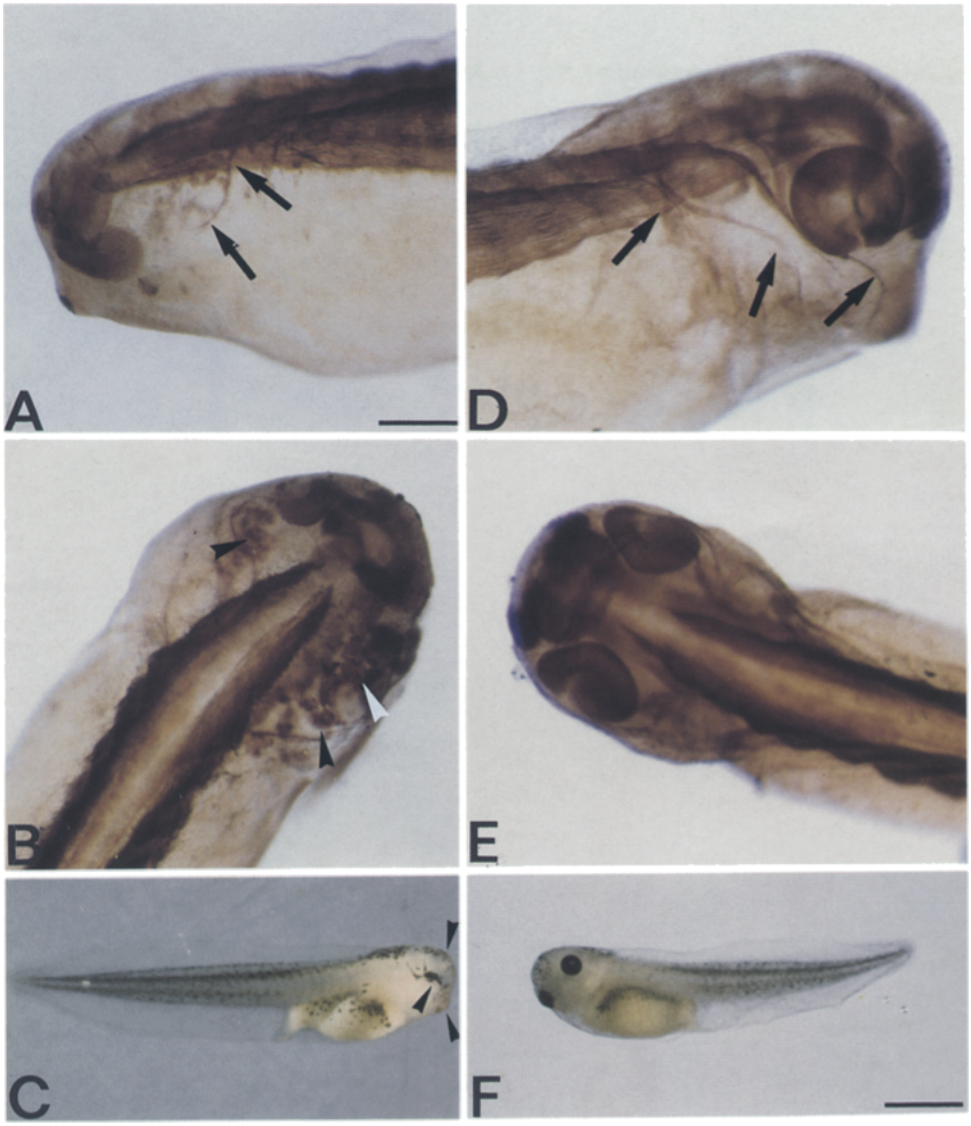


Figure 4. Perturbations produced by injection of pΔNXBcad mRNAs at 1× concentration. Whole-mount immunostaining of an injected (*A* and *B*) and control (*D* and *E*) embryo at the stage 32NF with the anti-N-CAM 4D monoclonal antibody and the 12/101 monoclonal antibody directed against skeletal muscle. *A* and *D* are the lateral views, and *B* and *E* are the dorsal views. Numerous N-CAM positive cell clusters are observed (*B*, *arrowheads*), as well as a delayed development of eye and of cranial nerve emergence (*A*, *arrows*) in the case of the injected embryos when compared to the control (*D*, *arrows*, and *E*). No defect in somitic development is detectable at this stage. Morphology of an injected (*C*) and control (*F*) embryo at stage 41NF. Abnormal development of anterior structure is visible in *C*, which represents a typical injected embryo. The main defect is observed on the eye development (*arrowheads*). Bar, 290 μm in *A*, *B*, *D*, and *E* and 640 μm in *C* and *F*.

mRNA was used at a concentration (1×) that gave rise to significant developmental defects, but that did not correspond to the maximum effect. Two independent experiments were performed where both pΔNXBcad and pΔNcad mRNA were coinjected each at a 1× concentration. Interestingly, the coinjection of both pΔNXBcad and pΔNcad mRNA at this concentration produced developmental defects at very high frequency, 92.3% (n = 13) when compared to that of individual injection at a concentration of 1× or 5×. This result indicated that the truncated cadherin species had additive effects in generating developmental abnormalities.

Grades of Perturbations Induced by the Expression of Truncated Cadherins

Injected embryos fixed at stage 41NF were sectioned, stained using the Ramon y Càjàl trichrome procedure, and analyzed to define grades of perturbations. The nature of the perturbation appeared to be strongly dependent on the type and amount of mRNA injected. In this study, we focused on the analysis of pertubation in the retina because even minor defects can be easily detected. Injected pΔNXBcad mRNA at 1× and 5× concentrations resulted in strong defects in the anterior nervous system characterized by incomplete optic cup morphogenesis (the cup most frequently remained fused to the floor of the diencephalon; Fig. 6 *G*, *arrowheads*, *J*, and *K*), as well as disorganization in retinal cell layers (Fig. 6, *J*, *H*, and *I*, *arrows* and *arrowheads*). 44.7% and 50% of the embryos analyzed presented this phenotype for 1× and 5× concentrations, respectively (Fig. 5 *B*).

When low amounts of pΔNXBcad mRNA (0.1× concentration) or higher amounts of pΔNcad mRNA (1× and 5× concentrations) were injected, the prominent abnormalities observed were a disorganization of the retinal layers; these defects were observed in 17.7%, 16.7%, and 17.4% of the embryos analyzed, respectively (Fig. 5 *B*). Defects in the morphogenesis of the optic cup were also visualized (0%, 3.3%, and 17.4%, respectively). For pΔNcadNCAM mRNA injections at the 1× and 5× concentrations, all the perturbed embryos (9% (n = 12) and 16% (n = 25) of injected embryos, respectively), presented only defects in the organiza-

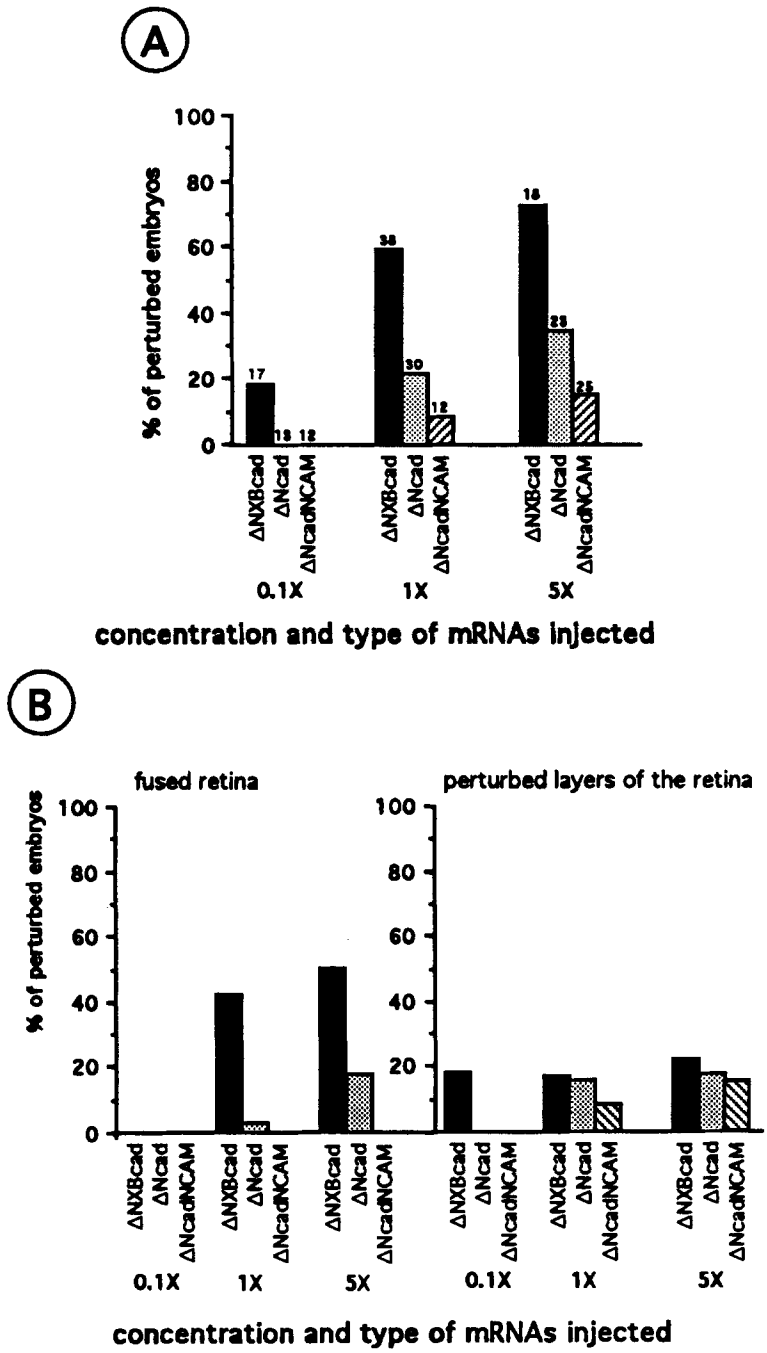
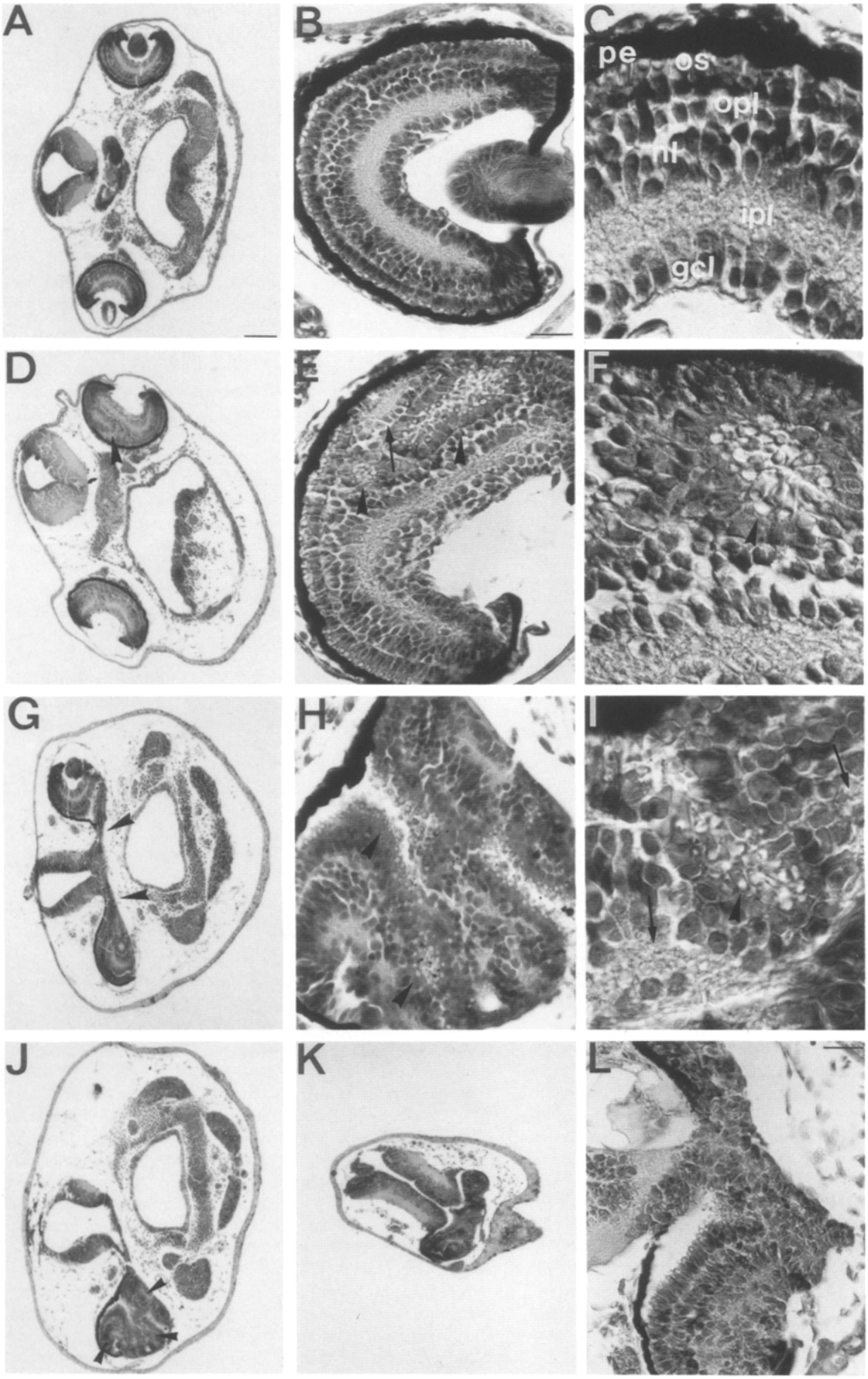


Figure 5. (*A*) Quantification of the perturbations obtained for the different truncated cadherins: the results are expressed in percent of embryos with malformations for increased concentrations of mRNA (0.1×, 1 ×, and 5× concentrations corresponding to 0.025, 0.25, and 1.25 ng of mRNA per blastomere, respectively). Increased concentrations of mRNA induce more severe malformations in anterior structures. (*B*) Grades of perturbations induced by the injection of the different mRNA. The grades were scored by determining the percent of perturbed embryos showing a perturbed morphogenesis of the retina that remains fused to the central nervous system (*left panel*) or a disorganization in the retinal layers (*right panel*). pΔNXBcad mRNA at the 1× and 5× concentrations primarily induced severe developmental defects of anterior structures, with the retina remaining attached to the floor of the diencephalon. In contrast, similar concentrations of pΔNcad mRNA induced mainly abnormalities in the organization of the plexiform layers of the retina. Embryos injected with pΔNcadNCAM mRNA exhibit only perturbations in the organization of the retinal layers.

Figure 6. Ramon y Càjàl trichrome histological staining of stage 41NF embryos. Histologically stained section of a control embryo (*A*) showing, from left to right, the brain with an eye on each side and the pharynx. (*D*) Typical embryo showing perturbations in retinal cell layers (*arrowhead*). The mesencephalon and the pharynx have a normal morphology and localization, while the organization of the retinal layers are altered with additive photoreceptor and neuropil clusters clearly observed at higher magnification (*E* and *F, arrowheads and arrows*). (*G, J,* and *K*) Another type of perturbation observed after injections of mRNA coding for truncated NXB-cadherin at 1× or 5× or N-cadherin at 5× concentration. Optic vesicles exhibit strong defects in their morphogenesis with retinas remaining fused to the ventral part of the brain (*G, arrowheads*). They either exhibit additive photoreceptor and neuropil clusters (*J, arrowheads*) or more drastic perturbations with delayed and aborted morphogenesis of the eye (*K*). Morphology of a control eye (*B* and *C*, at higher magnifications). From left to right on *B* and from top to bottom on *C*, the pigmented retinal epithelium (*pe*), the outer segment of the photoreceptors (*os*), the nuclear layer (*nl*), the plexiform layer (*pl*), and the ganglion cell layer (*gcl*) can be identified. Higher magnifications of a representative retina showing perturbation in its cell layer organization (*E* and *F*). Note a neuropil clustering near the photoreceptor layer (*arrow*) and an additional photoreceptor cell clustering in the nuclear layer (*arrowheads*). Higher magnification of fused retinas (*H, I,* and *L*) showing also stronger alterations of the eye structure represented by multiple small retina arrangements composed of all cell layers that were juxtaposed into the same eye cup (*H* and *I*, neuropil layer, *arrows*; double-photoreceptor layer, *arrowhead*). Bar, 100 μm in *A, D, G, J,* and *K*, 23 μm in *L*, 20 μm in *B, E* and *H*, and 10 μm in *C, F* and *I*. *C* and *F* are made with the Nomarski optical system.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tion of the retinal cell layers. We never observed fused retinas, even for the 5× concentration pΔNcadNCAM mRNA injection.

The perturbed retina often exhibited isolated plexiform layer clusters near the pigmented epithelium (Fig. 6 *E*, *arrows*), and groups of photoreceptor cells inserted into the nuclear cell layer (Fig. 6, *D–F*, *arrowheads*). Section of the anterior portion of control embryos injected with β-galactosidase mRNA was shown in Fig. 6 *A*. Corresponding retina at different magnifications was shown in Fig. 6, *B* and *C*. These structures never exhibited abnormalities in their development. In the case of embryos coinjected with pΔNXBcad and pΔNcad mRNA at the 1× concentration, we observed a fused or aborted retina in 84.6% of analyzed embryos, and only abnormalities in the retinal layers were observed in 7.7% of the embryos analyzed (data not shown).

The retinal masses of control and perturbed embryos (according to the type of perturbation) were quantified at stage 41NF. In the case of control embryos, injected embryos exhibited perturbed retinal layers, or injected embryos showed a fused retina, the mean of retinal mass was 5.94 ± 0.3, 6.25 ± 1.2, and 3.90 ± 1.8 (n = 8, arbitrary units), respectively. Control embryos represented a homogenous population concerning their retinal mass. A small increase of retinal mass was observed for embryos exhibiting abnormalities in their retina, which represented hyperplasia caused by additive cell layers such as photoreceptor layers or neuropil clusters, and was observed for other anterior structures later in the development of injected embryos. Embryos exhibiting fused retinas constituted a more heterogenous population that corresponded to distinct types of fusion. The first type showed a developmental timing and retinal mass values similar to the control eye or an eye presenting hyperplasia (Fig. 6, *G* and *J*). The second type, which exhibited mainly lower retinal mass, corresponded to embryos presenting a delayed eye morphogenesis (Fig. 6 *K*).

Early Morphogenetic Defects

Injection of pΔNXBcad mRNA in the blastomeres A1 and A2 also lead to severe malformation in the eye cup of stage 23NF embryos, as observed for injections into the four animal dorsal blastomeres. The descendants of these blastomeres can be followed by coinjecting β-galactosidase mRNA. Moderately labeled β-galactosidase–positive cells are seen in the diencephalon, in the neural retina anlage, and in the cement gland; most of these cells do not have a radial organization (Fig. 7 *A*, *arrowheads*). Heavily labeled cells are regrouped in clusters outside the eye cup (Fig. 7 *A*, *arrows*). All the neuroepithelial cells and the clustered cells are N-CAM positive (Fig. 7 *B*, *arrows*). The truncated cadherin can be easily immunolabeled in the clustered cells by the anti-tag antibody (Fig. 7 *C*); however, the truncated cadherin cannot be routinely detected by this method in the disorganized neuroepithelial cells that express β-galactosidase moderately. It is likely that the injected mRNA are not uniformly distributed in all the progeny of the injected blastomeres and/or that the enzymatic activity is a more sensitive method than immunolabeling in this particular case. pΔNcadNCAM mRNA injected in the same blastomeres did not induce any malformation; well-organized radial neuroepithelial cells are immunolabeled with the anti-tag antibody (Fig. 7 *D*).

Differential Alteration of Adhesive Contacts at Early Stages

To better understand the differential effect of the two different truncated N- and NXB-cadherins, we have searched for the earliest detectable pertubation events. After injection of pΔNcad and pΔNXBcad mRNA into the 4-AD blastomeres at the 32-cell stage, the first phenomenon observed was a release of cells in the anterior part of the embryo at stage 13-13.5NF. This cell release was analyzed for embryos injected at the 32-cell stage with 0.25 ng of pΔNXBcad mRNA (1× concentration) using a time-lapse video. At stage 12.5NF, some cells in the anterior part of the embryos started to protrude extensively in a limited area. This was followed by a spilling over of cells, apparently coming from the inner cell layer of the anterior ectoderm. Some pigmented cells from the outer cell layer of the embryo were also released at that time. The entire process of cell release, which lasted ~45 min, was initiated when the embryo was undergoing two contractions, as seen by video analysis. The cell release resulting from the inhibition of cadherin mediated cell–cell adhesion caused by the truncated cadherin expression is a good indicator for reduced adhesive strength. However, the number of cells released (around several tens or hundreds of cells released) is small compared to the estimated number of cells originating from the 4-AD blastomeres at stage 13NF (~3,000 cells). The results are presented in Table I; for identical amounts of mRNA, the number of cells released was higher with pΔNXBcad than with pΔNcad. At a 5× concentration, 52.8% of the pΔNXBcad embryos and 14.8% of pΔNcad embryos exhibited an "hn" phenotype (several hundreds of cells expelled) and 30.5% and 3.7% for the "ln" phenotype (several tens of cells expelled). At the intermediate mRNA concentration (1×), similar differences in the ability of two mRNA species to produce hn and ln phenotypes were observed. In contrast, at lower concentration (0.1×), neither pΔNXBcad nor pΔNcad induced the hn phenotype, and both promoted identical low levels of ln embryos (5.9% and 4%). Cell release was never observed when control pΔNcadNCAM mRNA was injected, even at the 5× concentration.

Truncated Cadherin-Catenin Interactions

To check the ability of our truncated cadherin to interact with catenins, we performed immunoprecipitation experiments using the P5D4 monoclonal antibody on cell extracts prepared from embryos coinjected with mRNA and ^{35}S-Trans-label mixture. The truncated N- and NXB-cadherins contain the same extracellular domain carrying the VSVG-16 epitope specifically recognized by this monoclonal antibody. The truncated N-cadherin (Fig. 8 *A*) and NXB-cadherin (Fig. 8 *B*) are able to coprecipitate the three known α-, β-, and γ-catenins (*arrows*). The autoradiography was scanned, and the band intensity value corresponding to each truncated cadherin and catenin was calculated. The ratio between the band intensity of the truncated cadherin and β-catenin was determined. The ΔNcad/β-catenin and ΔNXBcad/β-catenin ratios were 1.6 and 0.75, respectively. This suggests that the cytoplasmic domain of ΔNXB-cadherin has more affinity for β-catenin than that of ΔN-cadherin, and it might account for the stronger perturbation effects obtained from the truncated NXB-cadherin expression on embryonic development.

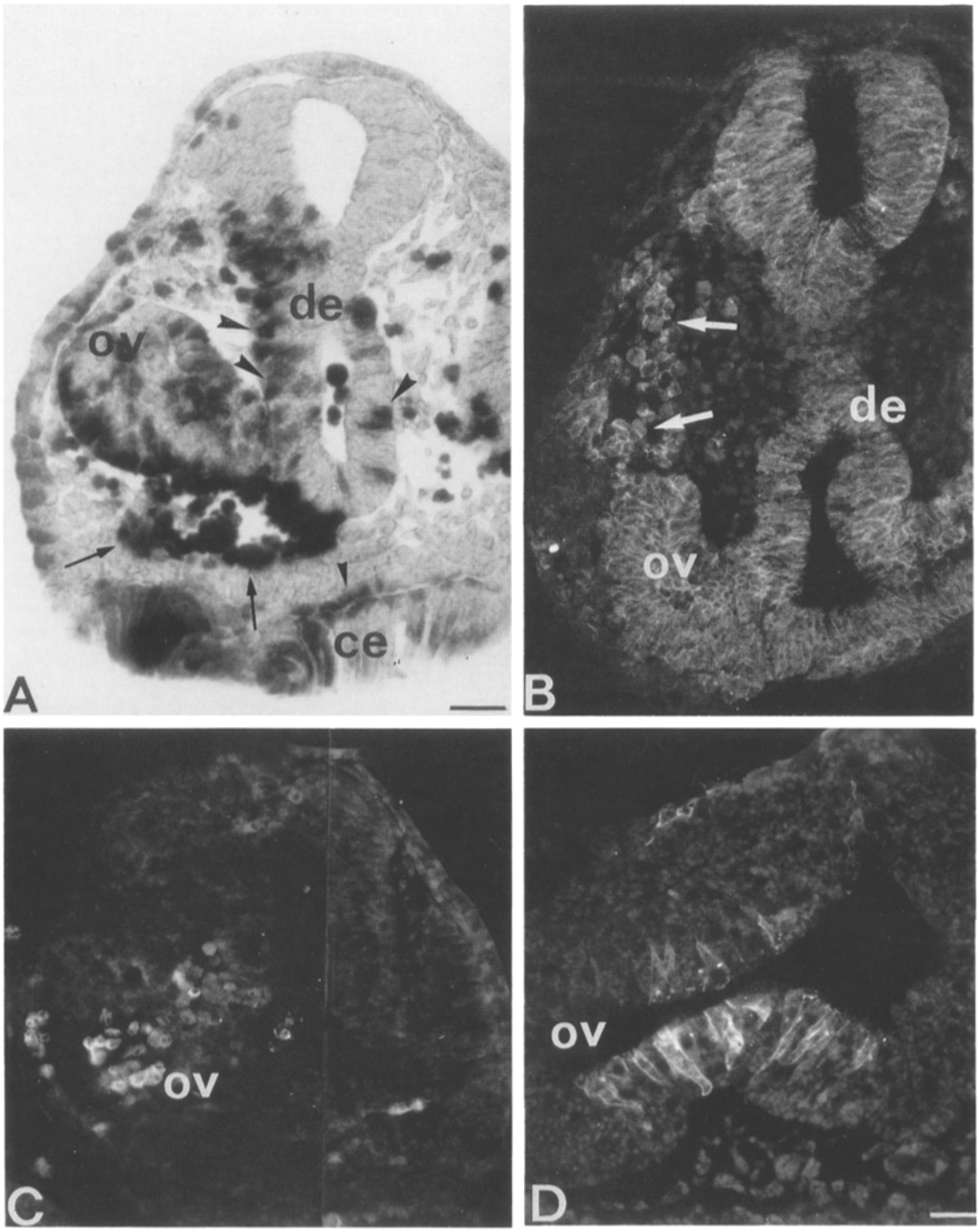

Figure 7. Sections of a stage 23NF embryo injected with pΔNXBcad mRNA and β-galactosidase mRNA (*A*), with pΔNXBcad mRNA (*B* and *C*), or with pΔNcadNCAM mRNA (*D*). In *A*, the embryo was processed for X-gal staining before sectioning. The sections are stained with the anti-N-CAM polyclonal antibody (*B*), and with the P5D4 monoclonal antibody directed against the epitope "tag" (*C* and *D*). (*A*) Many βgal-positive cells are seen in the diencephalon, in the optic vesicle, and in the cement gland. Note that these cells do not have a radial arrangement (*arrowheads*), as compared to their unlabeled or very weakly labeled neighbors. Most strongly labeled βgal-positive cells are found as clusters at the vicinity of the eye cup (*arrows*), a few are also seen in the lumen of the central nervous system. (*B*) These cells (*arrows*), as well as all the neuroepithelial cells, express N-CAM. (*C*) The truncated cadherin was mostly detected in the intensely labeled βgal-positive cells outside the optic cup. (*D*) The truncated N-CAM is expressed in a number of well-organized neuroepithelial cells. Bar, 50 μm in *A*, *B*, and *C*, and 30 μm in *D*. *de*, diencephalon; *ov*, optic vesicle; *ce*, cement gland.

Table I. Quantification of Cell Dissociation after Injection of the Different mRNAs

RNA injected	Number of cases	Percent of embryos with cell release (ln)	Percent of embryos with cell release (hn)
ΔNXBcad5×	36	30.5	52.8
ΔNXBcad1×	47	17	19.2
ΔNXBcad0.1×	34	5.9	0
ΔNcad5×	27	3.7	14.8
Δcad1×	68	13.2	1.5
ΔNcad0.1×	25	4	0
ΔNcadNCAM5×	25	0	0
ΔNcadNCAM1×	12	0	0

The 0.1×, 1×, and 5× concentrations correspond to 0.025 ng, 0.25 ng, and 1.25 ng of mRNA injected per blastomere, respectively. *ln*, one to several tens of cells released; *hn*, one to several hundreds of cells released.

Discussion

In the present study, we have generated cDNA constructs coding for the XB- and N-cadherins truncated in their extracellular domains to analyze the roles of these molecules during early embryogenesis in *Xenopus laevis*. A control construct consisted as a hybrid between N-cadherin and N-CAM in which the transmembrane and cytoplasmic sequences of the truncated N-cadherin were replaced by those of *Xenopus* N-CAM. The mRNA encoding these truncated molecules were injected into the four animal dorsal blastomeres of 32-cell stage embryos. We have observed the following: (*a*) During neurogenesis, the injected embryos display abnormal development of anterior structures, particularly the retina. (*b*) Different grades of pertubations are obtained after pΔNXBcad or pΔNcad mRNA injections. The pΔNXBcad mRNA injections preferentially induced abnormal optic cup morphogenesis, whereas pΔNcad mRNA injections affect mainly the organization of the retinal layers. (*c*) The truncated XB- and N-cadherins have additive effects in generating developmental abnormalities. (*d*) The cells expressing high levels of the truncated cadherins segregate from the neural tissue and are found as clusters in the head mesenchyme. They coexpress N-CAM and remain as aggregates in the head during later development. (*e*) The cytoplasmic domain of truncated NXB-cadherin seems to bind more efficiently to β-catenin than that of truncated N-cadherin.

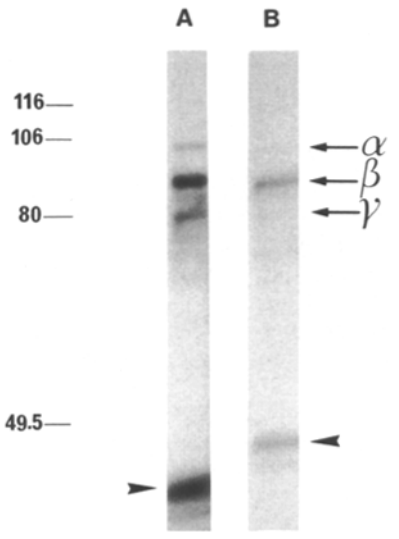

Figure 8. Catenins interact with truncated cadherins in injected embryos. mRNA were coinjected with ^{35}S-Trans-label mixture at the two-cell stage of *Xenopus* embryos. At stage 13-13.5NF, the embryos were homogenized in SDS-free immunoprecipitation buffer and incubated with P5D4 monoclonal antibody. The radiolabeled proteins immunoprecipitated were loaded on 7.5% acrylamide gel and revealed by autoradiography. The catenins (*arrows*) coprecipitating with the truncated N-cadherin and NXB-cadherin (*arrowheads*) were shown respectively on lanes *A* and *B*. Molecular weight markers were indicated on the left.

Truncated Cadherins Induced Developmental Defects in Anterior Structures

XB- and N-cadherin were chosen here because they display specific and different expression patterns during early development of the nervous system. XB-cadherin is already expressed at the blastula stage (Herzberg et al., 1991). Later, its expression declines in the nervous system, while it appears pronounced in the cement gland and becomes restricted to different epithelia (Wedlich, D., unpublished results). N-cadherin appears in the neuroectoderm after neural induction (Detrick et al., 1990; Simmoneau et al., 1992). This molecule is mainly expressed in the brain, placodes, retina, and some mesodermal derivatives during later development.

In contrast to previous studies, we have restricted the site of injection of the truncated cadherin mRNA to the four blastomeres of the 32-cell stage that contribute extensively to anterior neural structures (Dale and Slack, 1987; Jacobson and Hirose, 1981; Moody, 1987). Injection of mRNAs coding for truncated cadherin induces cell release at stage 13NF in the ectodermal cell layers and generates different abnormalities during later development. We have observed through the morphological appearance of the injected embryos and through whole-mount immunostaining performed with a monoclonal antibody against N-CAM that significant malformations of the anterior neural structures during early development existed without any distinguishable alteration of other structures. The malformations are restricted to the tissues derived from the injected blastomeres.

The expression of truncated NXB-cadherin gives rise to pronounced defects in anterior neural structures occurring though a relatively small number of cells were expelled at earlier stage. The expression of a truncated N-cadherin resulted primarily in small defects such as alterations in the organization of the cell layers of the retina. These effects cannot be solely explained by differences in the number of cells released at the earlier stages, but can be explained mainly by the fact that cells expressing the truncated cadherins perturb further development of the nervous system and other cranial structures. This is caused by their altered adhesive properties that do not permit their ordered assembly with nonperturbed cells into each tissue. Several kinds of perturbation experiments have shown that morphogenesis cannot easily be perturbed by the loss of cells or the arrest of cell division. For example, at the 32-cell stage, bilateral ablation of the A1 blastomere, which contributes to 50% of the retina, still results in a majority of tadpoles with either nor-

mal or reduced-size well-differentiated retina (Huang and Moody, 1993). On the other hand, the surgical removing of half of the embryonic eye bud including the proliferative zone portion (Wetts et al., 1993) gives rise to normal eye morphogenesis and structure with only a reduction in the final size. It was also observed that the blocking of cell division by hydroxyurea and aphidicolin, as early as stage 10NF, did not prevent morphogenesis and differentiation to occur in the nervous system, although it contains many less cells (Harris and Hartenstein, 1991).

Anterior neural tissues are always present in our injected embryos, even if they appear to be abnormal in their organization. Therefore, as previously observed in blastomere ablation experiments (Huang and Moody, 1993), blastomeres other than the four animal dorsal can contribute to the anterior neural system. In our case, the possible contribution of other blastomeres than those injected to give rise to nervous tissue appears to be not sufficient to allow a proper rescue of aberrant neural structures. Eye morphogenesis is one of the targets of the pertubation, and it is dependent on the blastomeres injected and on the type of mRNA used. These defects are sometimes associated with hyperplasia of other neural structures, such as the brain and the olfactory placodes, particularly when mRNA coding for other truncated cadherins were injected (Broders, F., J. M. Girault, and J. P. Thiery, manuscript submitted for publication).

Anterior Defects Appear Only at the Early Neurula Stage

The primary target proteins in competition with truncated cadherins for catenin binding might be represented by the maternal cadherins. One would expect that the function of XB-cadherin and EP-cadherin should be affected equally by injection of pΔXBNcad mRNA because these isoforms share a sequence similarity of 93% in their cytoplasmic domain. In comparison to their expression pattern (Angres et al., 1991), the perturbations appear relatively late in development when E-cadherin is also present. XB-cadherin and EP-cadherin are abundant, maternally derived proteins, and they are expressed de novo during midblastula transition. Besides this store of cadherins, α- and β-catenins are also found maternally expressed and in particular free α-catenin is in excess during early embryogenesis (Schneider et al., 1993). This relative high concentration of cadherins and catenins might impede successful competition by truncated cadherins at earlier stages (blastula/gastrula). The observed perturbations at the neurula stage correlate with the declining expression of XB- and EP-cadherins (Ginsberg et al., 1991; Levi et al., 1991*a*) and the decrease of free α-catenin (Schneider et al., 1993), while the truncated cadherins are extremely stable at least until stage 25NF.

Differential Effect of Cytoplasmic Domains of Cadherins and Possible Mechanisms

At the equivalent of stage 13NF, the truncated cadherins induced disruption of intercellular contacts in the ectodermal layers of the embryos. In our hands, the truncated N-cadherin was able to induce dissociation at stages where the endogenous N-cadherin is not yet expressed (Detrick et al., 1990), suggesting, as previously reported (Kintner, 1992), that it can interfere with the early cadherins such as XB-, U-, and E/P-cadherins (Angres et al., 1991; Ginsberg et al., 1991; Herzberg et al., 1991; Levi et al., 1991*a*). Interestingly, the extent of cell release depends essentially on the type and concentration of mRNA injected. The truncated NXB-cadherin is more efficient than the truncated N-cadherin in inducing cell disruption, and this is consistent with the presence of this molecule at the stage at which cell dissociation is observed. The coinjection of both pΔNcad and pΔNXBcad mRNA at 1× concentration have additive effects in promoting cell release and developmental defects. In contrast, the control pΔNcadNCAM hybrid construct did not induce cell release. Besides the inability of the pΔNcadNCAM hybrid to induce cell release, the fact that pΔNcad and pΔNXBcad possess the same transmembrane domain clearly indicates that the disruptive effects are dependent on the cytoplasmic domain of these cadherins. The observation that the cytoplasmic domains of XB- and N-cadherin show distinct and additive disruptive activities could be explained by the fact that the cytoplasmic domains of the two cadherins share only 60% partial identity sequence. It is, therefore, possible that a given cadherin cytoplasmic domain competes specifically or more efficiently with the endogenous cadherin of the same type for the binding of catenins. Our truncated cadherins bind the three known α-, β-, and γ-catenins, keeping with the fact that these catenins do not exhibit specificity to different cadherins (Ozawa et al., 1989; Ozawa and Kemler, 1992; Herrenknecht et al., 1991). However, the truncated NXB-cadherin binds more efficiently β-catenin that the truncated N-cadherin. This suggests that distinct cadherin-cytoplasmic domains can differently titer this component, critical for the proper functioning of endogenous cadherins (McCrea et al., 1993; Peifer et al., 1993; Schneider et al., 1993), and can explain the differential embryonic defects obtained for pΔNcad and pΔNXBcad mRNA injected embryos. On the other hand, the titration of one of the components involved in the complex assembly of cadherins and catenins, into a higher order structure, may be also more effective with one truncated cadherin species (Kemler, 1993; McCrea et al., 1993). However, we did not observe specific components selectively coprecipitated by N- or NXB-truncated cadherin. We cannot exclude the possibility that the truncated cadherins also compete for the occupancy of natural sites, where the endogenous cadherins are found, as previously reported in an in vitro assay (Fujimori and Takeichi, 1993), or that other unidentified cytoplasmic ligands might exist that specifically bind to a given cadherin molecule that could not be detected by classical biochemical approaches.

Other Adhesion Systems

Cells expressing high levels of truncated cadherins, as detected by the expression of the "tag" epitope, are mainly found in the vicinity of the neural tissue. These cells still express N-CAM, indicating that they are capable of acquiring neural characteristics and furthermore that N-CAM is not sufficient to maintain these cells within the neural tissue. The N-CAM and cadherins, therefore, do not seem to complement each other, but they appear to be required to stabilize neural tissue integrity. This could reflect the additivity of adhesive forces operating between cells or, alternatively, the addition of distinct cytoplasmic signaling systems re-

quired for the function of the two modes of cell adhesion (Doherty et al., 1991). Expression of high level of the truncated NcadNCAM hybrid protein in *Xenopus* embryos can induce at low frequency embryonic defects of anterior structures. This hybrid carries the NCAM 140-kD cytoplasmic domain. A cytoplasmic ligand has not been described so far to interact with this cytoplasmic form of NCAM, whereas the cytoplasmic domain corresponding to the NCAM 180-kD form has been shown to interact with spectrin (Pollenberg et al., 1986, 1987). It would be interesting to investigate the effect of the expression of the 180-kD-truncated variant of NcadNCAM hybrid on cell adhesion and embryonic development. Furthermore, the coexpression of truncated cadherins and several types of NcadNCAM hybrids would allow a better understanding of NCAM cytoplasmic domain function and the possible cross-talk between these two cell-adhesion systems during embryogenesis.

The combination of the use of dominant-negative mutants of cadherins with the restriction of the sites of injection of these constructs provides evidence for the notion that their cytoplasmic domains play a role in the specificity of the adhesive functions of the cadherins. It is intriguing to consider the possibility that the cytoplasmic domain of cadherins bear specific functions to transduce signals leading to different morphogenetic events. Such a situation has been clearly established for several integrin cytoplasmic domains in cell migration, cell proliferation, and collagen gel contraction (Chan et al., 1992; Pasqualini and Hemler, 1994).

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