Experimental Cell Research **246,** 312–318 (1999)
Article ID excr.1998.4285, available online at http://www.idealibrary.com on IDEAL®

Reactivation of Delta–Notch Signaling after Injury: Complementary Expression Patterns of Ligand and Receptor in Dental Pulp

Thimios A. Mitsiadis,*,†,[1] Kaj Fried,‡ and Christo Goridis†

**IMEB EA 2198, Faculté d'Odontologie, Université de la Méditerranée, 13385 Marseille Cedex 5, France; †Laboratoire de Génétique et Physiologie du Développement, IBDM, CNRS/INSERM/Université de la Méditerranée/AP de Marseille, Campus de Luminy case 907, 13288 Marseille Cedex 9, France; and ‡Department of Neuroscience, Karolinska Institutet, S-171 77 Stockholm, Sweden*

The evolutionarily conserved Notch-mediated intercellular signaling pathway is essential for proper embryonic development of many tissues and organs. Recent data suggest that Notch receptors and their membrane-bound ligands Delta and Serrate are involved in both patterning and cell fate determination during odontogenesis. It remains, however, uncertain if Notch signaling is important for tooth homeostasis and regeneration. Here we report on the expression of Notch receptors and the Delta1 ligand in dental pulp of normal and injured adult rat teeth. Notch receptors were absent from normal adult dental tissues, whereas expression was upregulated after injury. In injured teeth, Notch2 was expressed in mesenchymal cells of the pulp both close to the site of injury (i.e., in the dental crown) and at a distance from it (i.e., in the dental roots), Notch3 expression was mainly associated with vascular structures, while Notch1 expression was restricted to few pulpal cells close to the lesion. None of them was expressed in odontoblasts. Expression of Delta1 was upregulated in odontoblasts of the injured teeth, as well as in vascular structures. These results demonstrate the reactivation of the Notch signaling pathway during wound healing and, furthermore, highlight the similarity between developmental and regenerative processes.

Key Words: **neurogenic genes; Notch; Delta; tooth; odontoblast; wound healing.**

INTRODUCTION

The Notch signaling pathway defines a fundamental cell fate control mechanism regulating multicellular development by governing the ability of undifferentiated precursor cells to respond to specific signals [1, 2]. Notch signaling has been conserved throughout evolution, and mutations in its components disrupt cell fate specification and embryonic development in numerous metazoan organisms [reviewed in 3–6]. This signaling pathway is best understood in *Drosophila*, where the *Notch* gene encodes a large cell surface receptor with an extracellular domain carrying multiple epidermal growth factor (EGF)-like repeats. Notch is known to interact with the membrane-bound ligands encoded by the *Delta* and *Serrate* genes. The extracellular domains of these ligands contain a variable number of EGF-like motifs and a cysteine-rich motif, referred to as the DSL (Delta–Serrate–Lag-2) domain. Ligand binding results in receptor activation, a process involving cleavage of the Notch protein [7–9] and interactions with cytoplasmic and nuclear proteins, such as the Deltex and the Suppressor of Hairless proteins [10, 11]. The Notch signaling pathway mediates local cell–cell interactions which enable adjacent cells to adopt different fates [2, 12]. In this process, the transient expression of Delta or Serrate on a cell among a group of equivalent cells ensures the commitment of this cell to differentiate and, at the same time, instructs the surrounding cells expressing Notch to adopt a different fate or to remain undifferentiated (mechanisms referred to as lateral specification or inhibition, respectively). Genes homologous to members of the *Drosophila* Notch signaling pathway have been cloned in mammals [reviewed in 5, 6, 13] and have been shown to be essential for normal development of many tissues and organs, such as the neural tube, somites, eyes, limbs, and thymus [14–20]. The general importance of Notch signaling is reinforced by findings that link mutations in human genes, which encode Notch ligands and receptors, to cancer [21, 22] and to two inherited human disease syndromes [23–25].

Recent data suggest that the Notch signaling pathway is also important for proper odontogenesis [26–28]. Teeth are organs that develop as a result of sequential and reciprocal interactions between the oral ectoderm and neural crest-derived mesenchyme. These interactions gradually transform the tooth primordia into complex structures with various cell types, among which the epithelial-derived ameloblasts and the mesenchyme-derived odontoblasts synthesize and secrete

[1] To whom correspondence and reprint requests should be addressed at IMEB EA 2198, Faculté d'Odontologie, 27 Blvd Jean Moulin, F-13385 Marseille Cedex 5, France. Fax: (+33) 4 9180 4343. E-mail: mitsiadi@ibdm.univ-mrs.fr.

0014-4827/99 $30.00

the organic components of the hard tissues of the teeth (the enamel and the dentin, respectively). During tooth development, expression of the Delta1 ligand is complementary to Notch expression in adjacent epithelial and mesenchymal cells and correlates with ameloblast and odontoblast differentiation [26, 28]. These results suggest that the cellular diversity of developing teeth is dependent on lateral specification mediated by Notch signaling. However, expression and possible roles of Notch receptors and ligands in normal and injured adult teeth have never been studied. Here we demonstrate upregulation of both the Notch receptors and the Delta1 ligand after injury. Surprisingly, in lesioned teeth, Notch2 expression was induced in mesenchymal cells rather far from the site of injury.

MATERIALS AND METHODS

Animals and tissue preparation. For studies on normal and injured adult teeth, four 12-month-old male Wistar rats (body weight 250–300 g) were used. Pulpal (Fig. 1A) and periodontal (Fig. 3A) lesions on the first left mandibular molars were made with a small bur on anesthesized animals which were then allowed to survive for 1 or 2 days. The animals were then deeply anesthetized with chloral hydrate (35 mg/100 g body wt) and perfused with Tyrode's solution, followed by a fixative containing 4% paraformaldehyde (PFA) and 0.2% picric acid in 0.1 M phosphate buffer. After perfusion, the mandible halves were dissected from the rest of the head, postfixed by immersion for 90 min in 4% PFA, and equilibrated with 20% sucrose buffer solution overnight. The tissues were then decalcified in 4% ethylenediaminetetraacetate (Titriplex, Merck, Germany) in cacodylate buffer for 6 weeks. Fourteen-micrometer cryostat sections were mounted on chrome alum/gelatin-coated glass slides and then processed for *in situ* hybridization and immunohistochemistry.

Probes, antibodies, in situ hybridization, and immunohistochemistry. For *in situ* hybridization studies, digoxigenin-labeled antisense and sense riboprobes for Delta1 [28] were synthesized following the manufacturer's instructions (Boehringer Mannheim). For immunohistochemistry, rabbit polyclonal antibodies against the extracellular domains of the mouse Notch1, Notch2, and Notch3 proteins [26] were used. These antibodies recognize rat Notch1, 2, and 3 with the same specificity. *In situ* hybridization and immunohistochemistry on cryosections were performed as previously described [26, 27]. Peroxidase was revealed by incubation with 3-amino-9-ethylcarbazole containing 1% H_2O_2.

RESULTS

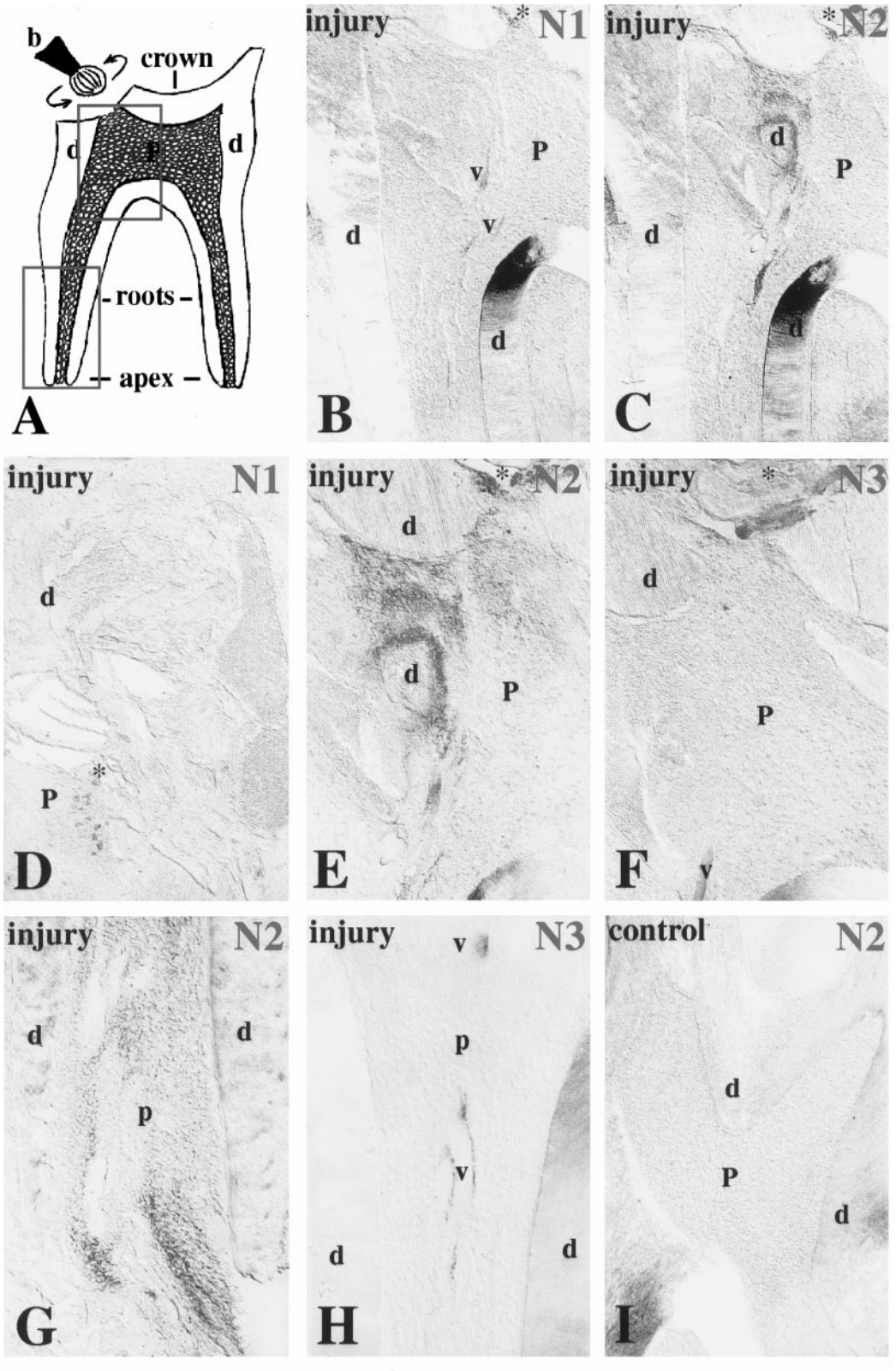
Localization of the Notch Receptors in Normal and Injured Adult Rat Teeth

Expression of the Notch receptors in sections of normal and injured rat molar teeth was analyzed by immunohistochemistry. In normal adult teeth, Notch1, 2, and 3 staining was absent from dental tissues (Figs. 1I and 2C and data not shown). In teeth with a pulpal lesion (Fig. 1A), immunoreactivity is observed for the three Notch proteins, but their expression patterns in the pulpal tissue were different. Notch1 was weakly expressed in a few mesenchymal cells of the tooth crown, close to the site of injury (Figs. 1B and 1D). A faint Notch1 staining was also found in vascular structures located in the crown pulp (Fig. 1B). Notch2 immunoreactivity was strong in the pulpal mesenchyme close to the lesioned area, while the staining was less intense in pulpal cells of the tooth crown at a distance from the injured area (Figs. 1C and 1E). Surprisingly, intense Notch2 staining was detected at sites far away from the injury such as in pulpal cells from distal parts of the roots (i.e., near the apex) (Figs. 1G, 2B, and 2E). Notch3 immunoreactivity was prominent in mesenchymal cells adjacent to the lesion (Fig. 1F). Although blood vessels traversing the crown pulp were negative for Notch3, a strong staining was found in vascular structures traversing the roots of the injured teeth (Fig. 1H).

Delta1 Expression in Lesioned Teeth: Comparison with Notch2 Expression

The terminal division of pulpal mesenchymal cells gives rise to two populations of cells with different developmental fates: odontoblasts, which are involved in dentin formation, and pulpal fibroblasts forming the connective tissue of the pulp. In adult teeth, odontoblasts form a layer with an epithelial appearance which serves as a protective barrier for the dental pulp. Odontoblasts are essential for the transfer of metabolites between pulp and dentin, and, furthermore, they may function as sensory receptors coupled to terminal

FIG. 1. Distribution of the Notch1 (N1), Notch2 (N2), and Notch3 (N3) proteins in injured (B–H) and normal (I) adult rat molars. Photomicrographs of avidin–biotin–peroxidase immunostaining on cryosections are shown. The site of injury is marked with an asterisk. (A) Schematic representation of the technique used to produce pulpal injuries. An occlusal surface cavity is made using a small rosehead bur (b) in a slow running handpiece until pulp (p) was barely exposed. (B) The region shown corresponds to the upper framed area (at the level of the crown) in (A). Weak N1 immunoreactivity is detected in pulpal cells close to the injury and in some vascular structures (v) 24 h after the injury. The black color in dentin (d) is an artifact due to light diffraction when the slides are viewed through Nomarski optics. (C) In the same framed area, strong N2 staining is found in pulpal cells close to the injury and in cells of the pulp chamber. Note the strong immunoreactivity around dentin debris that dropped into the pulpal chamber during the experimental procedure. (D) Higher magnification of (B), showing weakly N1-positive pulpal cells close to the injury. (E) Higher magnification of (C), showing pulpal cells expressing the N2 protein. Note the decreased staining at sites far away from the injured area. (F) Strong N3 immunoreactivity is found in pulpal cells close to the injury, as well as in vascular structures. (G) Lower framed area (at the root level) of the A. Intense N2 staining is detected in pulpal cells at the apical part of the root. (H) N3 immunoreactivity is expressed in blood vessels irrigating the roots. (I) N2 staining is absent from pulpal cells in the normal adult teeth. Bar, (B, C, I) 100 μm; (D–H) 60 μm.


b
crown
d
P
d
roots
apex
A
injury
N1
P
v
v
d
d
B
injury
N2
d
P
d
d
C
injury
N1
d
P
*
D
injury
N2
d
d
P
E
injury
N3
d
P
F
v
injury
N2
d
d
p
G
injury
N3
v
p
v
d
d
H
control
N2
d
P
d
I

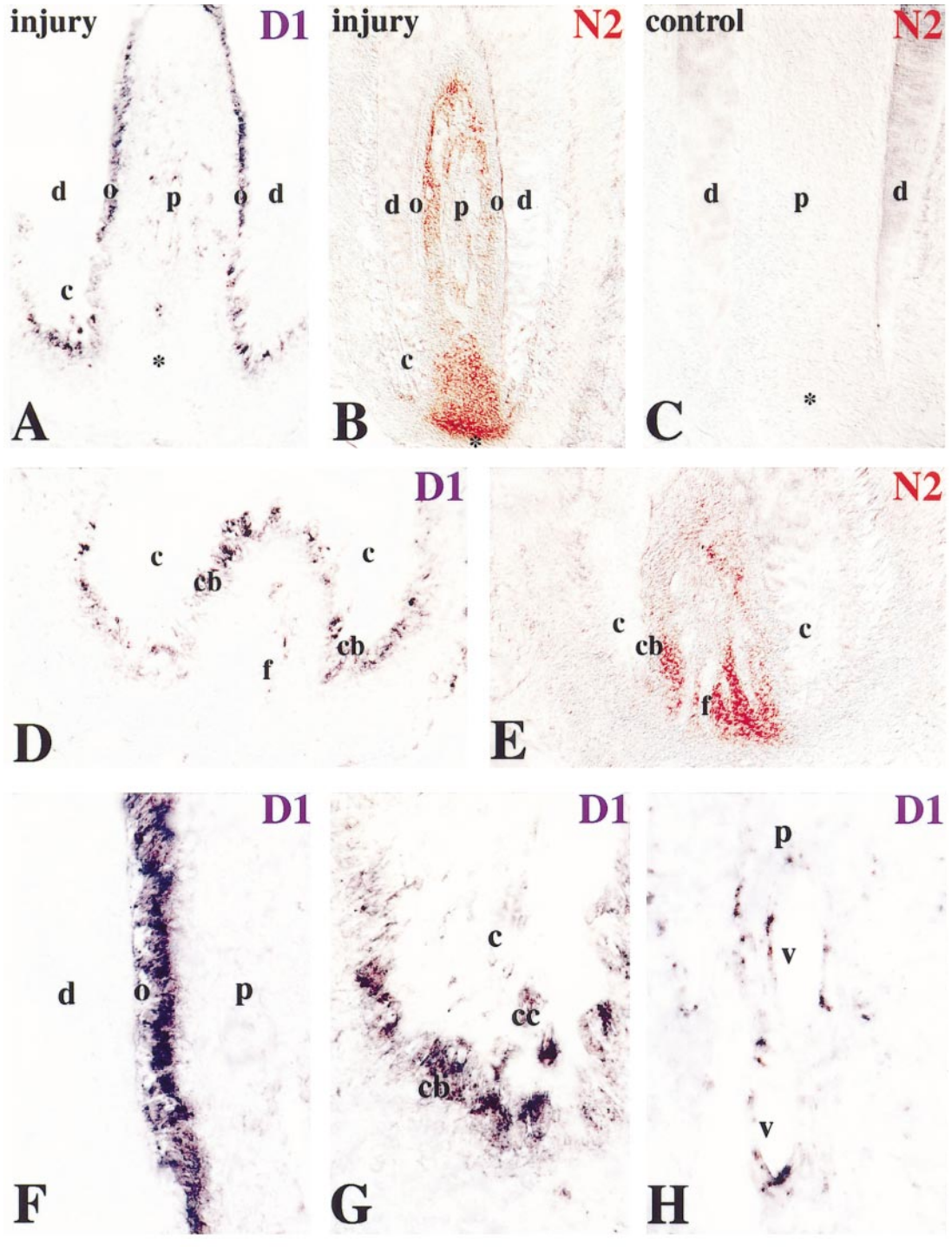


FIG. 2. Comparison between *Delta1* (D1) mRNA expression and expression of the N2 protein in injured rat teeth. Photomicrographs of *in situ* hybridization and immunostaining on cryosections with a digoxigenin-labeled probe and an anti-N2 antibody, respectively, are shown. The *in situ* signal is shown in violet (A, D, F–H) and the immunolabeling is shown in red (B, C, E). The region shown corresponds to the lower framed area in Fig. 1A. (A) Intense D1 expression is observed in odontoblasts (o) of the root, 24 h after the injury. Note that the D1 signal is also found in cells forming the cementum (c) of the apical root region (asterisk). (B) In the same area, N2 immunoreactivity is strong in pulpal cells (p), but the staining is absent in odontoblasts. Note that the N2 signal is very intense in mesenchymal cells of the apical root region (asterisk). (C) N2 staining is absent from cells of the apical root region in normal adult teeth. (D) In the apex of an injured tooth, the D1 signal is confined to the cementoblasts (cb). (E) In the same area, N2 staining is only observed in mesenchymal cells other than cementoblasts. (F, G) Higher magnification of the proximal (F) and apical (G) root regions of an injured tooth showing the intense D1 mRNA expression in odontoblasts (F) and in both cementoblasts and cementocytes (cc) (G). (H) Higher magnification of the root region showing D1 transcripts in blood vessels (v). Additional abbreviations: f, dental follicle; d, dentin; c, cementum. Bar, (A) 85 μm; (B) 100 μm; (C–E) 60 μm; (F–H) 35 μm.

nerve fibers. *Delta1* transcripts were absent from dental tissues of normal adult teeth (data not shown). By contrast, an intense *Delta1* hybridization signal was detected in odontoblasts of the injured teeth (Figs. 2A and 2F). Furthermore, *Delta1* was strongly expressed in cementoblasts and cementocytes located at the apical part of the roots (Figs. 2A, 2D, and 2G). Cementoblasts are follicular cells lining the root surface and are responsible for formation of the cementum-matrix of the teeth, while cementoblasts trapped in lacunae within their own matrix are named cementocytes. The expression pattern of *Delta1* in the roots of injured teeth was complementary to that of Notch2, which was only expressed in mesenchymal cells other than odontoblasts (Figs. 2B and 2E). Finally, *Delta1* transcripts were also observed in blood vessels traversing the dental roots (Fig. 2H).

Notch Expression in Periodontal Lesions

In a similar kind of experiment, periodontal lesions were performed and sections were analyzed by immunohistochemistry. Periodontium is the mesenchyme-derived tissue that links the teeth to the alveolar bones of the jaws while at the same time permitting the teeth to withstand the considerable forces of mastication. The periodontium consists of cementum, periodontal ligament and part of the alveolar bone. In periodontal lesions (Fig. 3A), immunoreactivity was observed for the Notch1 and 2 proteins, but not for the Notch3 protein. Notch1 was weakly expressed in a few osteocytes of the alveolar bone (Fig. 3B), whereas a very strong Notch2 immunoreactivity was detected in cells of the periodontal ligament close to the site of injury and in scarce cells of the alveolar bone (Fig. 3C).

DISCUSSION

The Notch signaling pathway controls cell fate commitment during development of a wide range of tissues and organs throughout the animal kingdom [reviewed in 2, 4–6, 12]. In *Drosophila*, Notch and its ligands are expressed in cells which are not yet terminally differentiated and are absent from adult tissues with the exception of the ovaries and testes [2, 6], but there are few studies on Notch or Notch ligand expression in adult vertebrates. In previous work, we have studied the regulation and expression of the Notch receptors and ligands during tooth development [26–28]. Our results suggested that the Notch pathway plays a prominent role in the cell fate choices leading to the terminal differentiation of ameloblasts and odontoblasts, the enamel- and dentin-matrix-synthesizing cells of adult teeth. In the present study, we have examined the expression of three Notch receptors and of the Delta1 ligand in normal and injured adult molars. We found that normal adult molars were devoid of Notch receptor and Delta1 expression, but that their expression was reinduced within 24 h after injury, probably reflecting initiation of regenerative processes.

Undifferentiated mesenchymal cells of the pulp represent a pool of cells which can give rise to odontoblasts and pulpal fibroblasts. Fibroblasts are particularly numerous in the coronal portion of the pulp where they form and maintain the pulp extracellular matrix. In adult rodents, the dental pulp retains some ability of repair after pulpal injury [reviewed in 29]. When odontoblasts are lesioned, mesenchymal cells of the pulp differentiate into new odontoblasts, which will form the repair dentin. Injury also results in an inflammatory response within the pulp, and invading macrophages eliminate dead cells and debris and fight bacterial invasion in conjunction with other inflammatory cells.

The present data suggest that the Notch signaling pathway is involved in the dynamic processes triggered by pulpal injury. Notch upregulation in the lesioned pulp may represent an early molecular event in tissue repair processes, since expression is observed as early as 24 h after injury. Although the three Notch receptors are reexpressed after lesion with distinct expression patterns, Notch2 expression in pulpal cells was predominant. During the cytodifferentiation stages of embryonic tooth development, the strongest Notch expression by mesenchyme-derived cells was seen in the sub-odontoblastic layer that is in still undifferentiated cells which are probably committed to an odontoblastic fate [30]. Also during regeneration of lesioned molars, Notch2 expression may thus be activated in pulpal cells which, albeit still undifferentiated, are now engaged in a differentiation pathway leading to odontoblasts and/or pulpal fibroblasts. In this way, activation of the Notch pathway may ensure a continuous supply of progenitors committed to become odontoblasts. In line with this interpretation, two previous studies, in which Notch gene or protein expression was studied in adult mammalian tissues, also arrived at the conclusion that Notch expression is associated with cells which represent a proliferating precursor pool dedicated to a specific fate [22, 31]. However, we are not aware of another study which addresses the role of Notch receptors after injury or during regeneration.

A surprising result was that Notch2 expression is not only activated in pulpal cells close to the injury, but also at the apex of the roots, suggesting that these sites represent important cell pools from which different pulpal cell types will derive after injury. As suggested by the expression of the Delta1 ligand in cementoblasts and cementocytes of the apical root, injury of the tooth crown may also stimulate cement formation by the roots. Notch3 and *Delta1* expression was activated in vascular structures of the root, reflecting either ingrowth of new blood vessels or an inflammatory reaction.

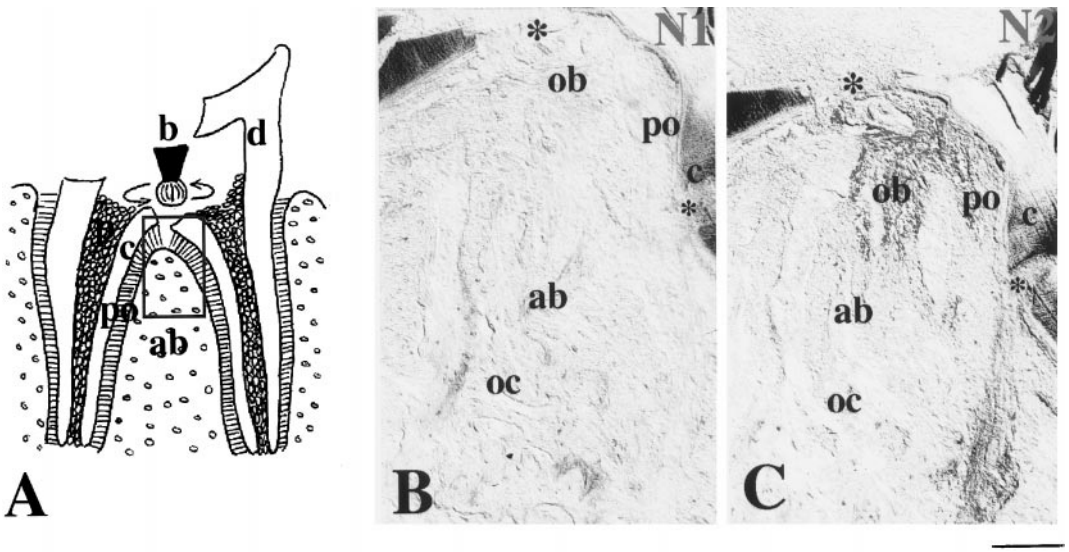


FIG. 3. Distribution of the N1 and N2 proteins in the periodontium of adult rat molars after injury. Photomicrographs of avidin–biotin–peroxidase immunostaining on cryosections are shown. The site of the injury is marked with an asterisk. (A) Schematic representation of the technique used to produce periodontal injury. The cavity is made at the bifurcation of the roots area using a bur (b) in a slow running handpiece until the periodontium (po) was exposed. (B) Framed area of A. Weak N1 immunoreactivity is detected in osteocytes (oc) of the alveolar bone (ab) 24 h after the injury. Note that the staining is absent from osteoblasts (ob) of the periosteum (po). (C) In the same area, strong N2 staining is found in osteoblasts of the periosteum close to the injury. Some osteocytes are also N2 positive. Additional abbreviations: c, cementum; d, dentin. Bar, 100 μm.

During odontogenesis, the *Delta1* and *Notch* genes show complementary expression patterns at several sites. For example, *Delta1* is expressed in differentiating odontoblasts, whereas *Notch* expression is confined to adjacent mesenchymal cells of the dental pulp [26, 28], suggesting a role for Delta–Notch signaling in the control of odontoblast differentiation. We found an analogous situation in injured molars, where *Delta1* is upregulated in odontoblasts and Notch2 is expressed only in pulpal cells other than odontoblasts. These results highlight the similarity between developmental and regenerative processes and support the notion that Notch activation is instrumental in maintaining a committed precursor pool for odontoblasts. However, we found Notch2 to be reexpressed in the pulp of the crown and in the roots also by cells which are not in contact with Delta1-expressing cells. In vertebrates, there are at least four different Notch ligands [13], and other Notch ligands may be expressed at these sites. Alternatively, Notch expression is initiated at several sites, but the pathway becomes activated only in cells in contact with the Delta1-bearing odontoblasts or cement-forming cells.

We also studied Notch and Delta1 expression in a similar experimental model focusing on periodontal lesions. The periodontal ligament is formed by dental follicle cells synthesizing its fibrillar components which are constantly being synthesized, removed, and replaced [29]. Expression of Notch receptors was found to be upregulated after injury in the periodontium. As in the injured pulp, Notch2 seems to be the main receptor involved in regenerative processes and its expression was confined to undifferentiated cells.

Although the molecular interactions underlying reparative processes are not well understood, signaling molecules of the TGFβ superfamily seems to be important to hard tissue formation after pulpal injury [32]. Furthermore, it has been shown that TGFβ1 and BMPs may induce odontoblast differentiation [reviewed in 33] and upregulate *Delta1* expression in dental mesenchyme *in vitro* [28]. These results suggest that members of the TGFβ superfamily may be also involved in regulating expression of Notch receptors and ligands after tooth injury.

A variety of studies have shown that deregulation of Notch signaling is associated with different types of neoplasias [21, 22, 34, 35]. We show here that elevated levels of Notch receptors and the Delta1 ligand are also associated with the physiological response to injury. Together, these data indicate that properly regulated activation of the Notch signaling pathway is important not only for controlling cell fate choices during development, but also for maintaining the correct balance between proliferation and differentiation and thus tissue homeostasis in the adult organism.

We are grateful to Dr U. Lendahl (CMB, Karolinska Institutet, Sweden) for the gift of the anti-Notch1, 2, and 3 antibodies. This work was supported by grants from the Fondation pour la Recherche Médicale (T.M.), the Association pour la Recherche sur le Cancer, the Ligue Nationale Contre le Cancer (T.M., C.G.), and the Swedish Medical Research Council (K.F.).

REFERENCES

1. Heitzler, P., and Simpson, P. (1991). The choice of cell fate in *Drosophila* neuroepithelium. *Cell* **64,** 1083–1092.
2. Artavanis-Tsakonas, S., Matsuno, K., and Fortini, M. E. (1995). Notch signaling. *Science* **268,** 225–232.
3. Artavanis-Tsakonas, S. (1997). Alagille syndrome-a notch up for the Notch receptor. *Nature Genet.* **16,** 212–213.
4. Nye, J. S., and Kopan, R. (1995). Vertebrate ligands for Notch. *Curr. Biol.* **5,** 966–969.
5. Gridley, T. (1997). Notch signaling in vertebrate development and disease. *Mol. Cell. Neurosci.* **9,** 103–108.
6. Weinmaster, G. (1997). The ins and outs of Notch signaling. *Mol. Cell. Neurosci.* **9,** 91–102.
7. Kopan, R., Schroeter, E. H., Weintraub, H., and Nye, J. S. (1996). Signal transduction by activated mNotch: Importance of proteolytic processing and its regulation by the extracellular domain. *Proc. Natl. Acad. Sci. USA* **93,** 1683–1688.
8. Blaumueller, C. M., Qi, H., Zagouras, P., and Artavanis-Tsakonas, S. (1997). Intracellular cleavage of Notch leads to a heterodimeric receptor on the plasma membrane. *Cell* **90,** 281–291.
9. Pan, D., and Rubin, G. M. (1997). Kuzbanian controls proteolytic processing of Notch and mediates lateral inhibition during *Drosophila* and vertebrate neurogenesis. *Cell* **90,** 271–280.
10. Fortini, M. E., and Artavanis-Tsakonas, S. (1994). The Suppressor of Hairless protein participates in Notch receptor signaling. *Cell* **79,** 273–282.
11. Matsuno, K., Diederich, R. J., Go, M. J., Blaumueller, C. M., and Artavanis-Tsakonas, S. (1995). Deltex acts as a positive regulator of Notch signaling through interactions with the Notch ankyrin repeats. *Development* **121,** 2633–2644.
12. Lewis, J. (1996). Neurogenic genes and vertebrate neurogenesis. *Curr. Opin. Neurobiol.* **6,** 3–10.
13. Lendahl, U. (1998). A growing family of Notch ligands. *BioEssays* **20,** 103–107.
14. Conlon, R. A., Reaume, A. G., and Rossant, J. (1995). Notch 1 is required for the coordinate segmentation of somites. *Development* **121,** 1533–1545.
15. Lardelli, M., Williams, R., Mitsiadis, T., and Lendahl, U. (1996). Expression of the Notch3 intracellular domain in mouse central nervous system progenitor cells is lethal and leads to disturbed neural tube development. *Mech. Dev.* **59,** 177–190.
16. Bao, Z.-Z., and Cepko, C. L. (1997). The expression and function of Notch pathway genes in the developing rat eye. *J. Neurosci.* **17,** 1425–1434.
17. de Angelis, M. H., McIntyre, J., II, and Gossler, A. (1997). Maintenance of somite borders in mice requires the Delta homologue Dll1. *Nature* **386,** 717–721.
18. de la Pompa, J. L., Wakeham, A., Correia, K. M., Samper, E., Brown, S., Aguilera, R. J., Nakano, T., Honjo, T., Mak, T. W., Rossant, J., and Conlon, R. A. (1997). Conservation of the Notch signalling pathway in mammalian neurogenesis. *Development* **124,** 1139–1148.
19. Sidow, A., Bulotsky, M. S., Kerrebrock, A. W., Bronson, R. T., Daly, M. J., Reeve, M. P., Hawkins, T. L., Birren, B. W., Jaenish, R., and Lander, E. S. (1997). Serrate2 is disrupted in the mouse limb-development mutant syndactylism. *Nature* **389,** 722–725.
20. Jiang, R., Lan, Y., Chapman, H. D., Shawber, C., Norton, C. R., Serreze, D. V., Weinmaster, G., and Gridley, T. (1998). Defects in limb, craniofacial, and thymic development in jagged2 mutant mice. *Genes Dev.* **12,** 1046–1057.
21. Ellisen, L. W., Bird, J., West, D. C., Soreng, A. L., Reynolds, T. C., Smith, S. D., and Sklar, J. (1991). Tan-1, the human homolog of the *Drosophila* Notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell* **66,** 649–661.
22. Zagouras, P., Stifani, S., Blaumueller, C. M., Carcangiu, M.-L., and Artavanis-Tsakonas, S. (1995). Alterations in Notch signaling in neoplastic lesions of the human cervix. *Proc. Natl. Acad. Sci. USA* **92,** 6414–6418.
23. Joutel, A., Corpechot, C., Ducros, A., Vahedi, K., Chabriat, H., Mouton, P., Alamowitch, S., Domenga, V., Cécillion, M., Maréchal, E., Maciazek, J., Vayssière, E., Cruaud, C., Cabanis, E.-A., Ruchoux, M. M., Weissenbach, J., Bach, J. F., Bousser, M. G., and Tournier-Lasserve, E. (1996). Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. *Nature* **383,** 707–710.
24. Li, L., Krantz, I. D., Deng, Y., Genin, A., Banta, A. B., Collins, C. C., Qi, M., Trask, B. J., Kuo, W. L., Cochran, J., Costa, T., Pierpont, M. E., Rand, E. B., Piccoli, D. A., Hood, L., and Spinner, N. B. (1997). Alagille syndrome is caused by mutations in human *Jagged1,* which encodes a ligand for Notch1. *Nature Genet.* **16,** 243–251.
25. Oda, T., Elkahloum, A. G., Pike, B. L., Okajima, K., Krantz, I. D., Genin, A., Piccoli, D. A., Meltzer, P. S., Spinner, N. B., Collins, F. S., and Chandrasekharappa, S. C. (1997). Mutations in the human Jagged1 gene are responsible for Alagille syndrome. *Nature Genet.* **16,** 235–242.
26. Mitsiadis, T. A., Lardelli, M., Lendahl, U., and Thesleff, I. (1995). Expression of Notch 1, 2 and 3 is regulated by epithelial-mesenchymal interactions and retinoic acid in the developing mouse tooth and associated with determination of ameloblast cell fate. *J. Cell Biol.* **130,** 407–418.
27. Mitsiadis, T. A., Henrique, D., Thesleff, I., and Lendahl, U. (1997). Mouse Serrate-1 (Jagged-1): Expression in the developing tooth is regulated by epithelial–mesenchymal interactions and fibroblast growth factor-4. *Development* **124,** 1473–1483.
28. Mitsiadis, T. A., Hirsinger, E., Lendahl, U., and Goridis, C. (1998). Delta–Notch signaling in odontogenesis: Correlation with cytodifferentiation and evidence for feedback regulation. *Dev. Biol.* **204,** in press.
29. TenCate, A. R. (1985). Development of the tooth and its supporting tissues. *In* "Oral Histology: Development, Structure and Function" Mosby, Princeton St. Louis Toronto.
30. Ruch, J.-V. (1987). A review: Determinisms of odontogenesis. *In* "Cell Biology Reviews" (E. Barbera-Guiltem, Ed.), Springer-Verlag, Berlin.
31. Kopan, R., and Weintraub, H. (1993). Mouse *Notch:* Expression in hair follicles correlates with cell fate determination. *J. Cell Biol.* **121,** 631–641.
32. Rutherford, R. B., Wahle, J., Tucker, M., Rueger, D., and Charette, M. (1993). Induction of reparative dentine formation in monkeys by recombinant human osteogenic protein-1. *Arch. Oral Biol.* **38,** 571–577.
33. Ruch, J.-V., Lesot, H., and Bègue-Kirn, C. (1995). Odontoblast differentiation. *Int. J. Dev. Biol.* **39,** 51–68.
34. Rohn, J. L., Lauring, A. S., Linenberger, M. L., and Overbaugh, J. (1996). Transduction of Notch2 in feline leukemia virus-induced thymic lymphoma. *J. Virol.* **79,** 8071–8080.
35. Pear, W. S., Aster, J. C., Scott, M. L., Hasserjian, R. P., Soffer, B., Sklar, J., and Baltimore, D. (1996). Exclusive development of T cell neoplasms in mice transplanted with bone marrow expressing activated Notch alleles. *J. Exp. Med.* **183,** 2283–2291.

Received July 29, 1998
Revised version received September 16, 1998