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Rapid Induction of the Major Embryonic α-Tubulin mRNA, Tα1, During Nerve Regeneration in Adult Rats

Freda D. Miller,[1,a] Wolfram Tetzlaff,[2] Mark A. Bisby,[2] James W. Fawcett,[3] and Robert J. Milner[1]

[1]Division of Preclinical Neurosciences and Endocrinology, Research Institute of Scripps Clinic, La Jolla, California 92037, [2]Department of Medical Physiology, University of Calgary, Calgary, Canada, and [3]Department of Physiology, Cambridge University, Cambridge, England

The mRNAs for 2 isotypes of α-tubulin, termed Tα1 and T26, are differentially regulated in the developing rat nervous system. Tα1 α-tubulin mRNA is expressed at high levels when neurons extend processes whereas T26 mRNA is expressed constitutively (Miller et al., 1987b). We have examined the expression of these 2 α-tubulin mRNAs in regenerating facial and sciatic motor neurons of the rat using Northern blot and *in situ* hybridization analyses. Tα1 α-tubulin mRNA is rapidly induced in axotomized motor neurons of the facial nerve: increased levels of mRNA are detectable 4 hr after a lesion is made 1.5 cm distal to the neuronal cell bodies. Tα1 mRNA levels are highest from 3–7 d postcrush and decline slowly to control levels following functional reinnervation of facial muscles. In contrast, T26 mRNA levels remain constant throughout the regeneration process. Total α-tubulin mRNA levels do not change until 1 d postaxotomy; otherwise the changes in expression are similar to Tα1 mRNA, although the relative increase is not as great. Enhanced Tα1 α-tubulin mRNA expression also occurs in motor neurons of crushed or tied sciatic nerve. Ligature or crush of the sciatic nerve leads to approximately the same peak in the expression of Tα1 mRNA at 7–15 d postaxotomy. Following the facial nerve transection, under conditions in which reinnervation is prevented, Tα1 α-tubulin mRNA levels remain elevated significantly longer than when the nerve is crushed. Taken together, the data indicate that Tα1 α-tubulin mRNA is rapidly induced following neuronal axotomy, remains elevated during the period of axonal regrowth, and is subsequently down-regulated at the approximate time of target contact. These results are reminiscent of changes in Tα1 mRNA that occur during neuronal development. This growth-associated pattern of Tα1 gene expression can be modified by inhibiting appropriate regeneration of the damaged nerve.

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Correspondence should be addressed to Dr. Freda D. Miller, Department of Anatomy and Cell Biology, University of Alberta, Edmonton, Alberta T6G 2H7, Canada.

[a] Present address: Department of Anatomy and Cell Biology, University of Alberta, Edmonton, Alberta, Canada.

Most vertebrate motoneurons respond to axonal injury by undergoing a series of well-defined morphological changes. The neuronal soma progresses through a complicated series of changes, including retraction of afferent synapses, establishment of new glial cell contacts, and reestablishment of appropriate afferent inputs. In addition, axons regenerate and form functional synapses with their targets. Microtubules, which are assembled from α and β-tubulins, are integral components of growing neurites (Daniels, 1972), both during development and during regeneration (Tetzlaff et al., 1988a). In mammals, at least 6 different α-tubulin genes (Villasante et al., 1986) and 5 different β-tubulin genes (Wang et al., 1986) are expressed in neural and non-neural tissues at various times during development. Of these, 2 distinct α-tubulin mRNAs (Ginzburg et al., 1981; Lemischka et al., 1981) and 3 β-tubulin mRNAs (Bond et al., 1984) are expressed in rat brain. Two α-tubulin mRNAs, Tα1 and T26, are expressed during embryogenesis (Miller et al., 1987b) and are homologous to Mα1 and Mα2 mRNAs that are expressed in an analogous manner in mice (Lewis et al., 1985). A third α-tubulin mRNA, Mα4, which has no known rat homologue, is also expressed in the postnatal mouse brain (Villasante et al., 1986).

The temporal and spatial patterns of Tα1 and T26 α-tubulin mRNA expression in the developing nervous system have recently been defined (Miller et al., 1987b). Tα1 mRNA is highly enriched in the embryonic nervous system, with expression being tightly coupled to neuronal process extension. In contrast, T26 mRNA is constitutively expressed in the nervous system as well as in other embryonic tissues. The correlation between Tα1 α-tubulin mRNA expression and neurite extension has been confirmed in PC12 cells treated with nerve growth factor (NGF) (Miller et al., 1987b) and in primary cultures of embryonic cortical neurons (M. Durand, F. D. Miller, and R. J. Milner, unpublished observations).

Since total tubulin protein levels increase following motoneuron axotomy (Tetzlaff et al., 1988a), it seemed reasonable to test whether the developmental patterns of tubulin isotype expression also occur in regenerating motoneurons. We therefore examined the expression of Tα1 and T26 α-tubulin mRNAs in unilateral crushes of the facial and sciatic nerves using a combination of Northern blot and *in situ* hybridization analysis. Data show that Tα1 α-tubulin mRNA is rapidly induced in motor neurons following axotomy and is eventually down-regulated following functional reinnervation. In contrast, T26 α-tubulin mRNA levels remain unchanged during neuronal regeneration. Tα1 α-tubulin mRNA expression is therefore tied to the extension of neuronal processes in the mature nervous

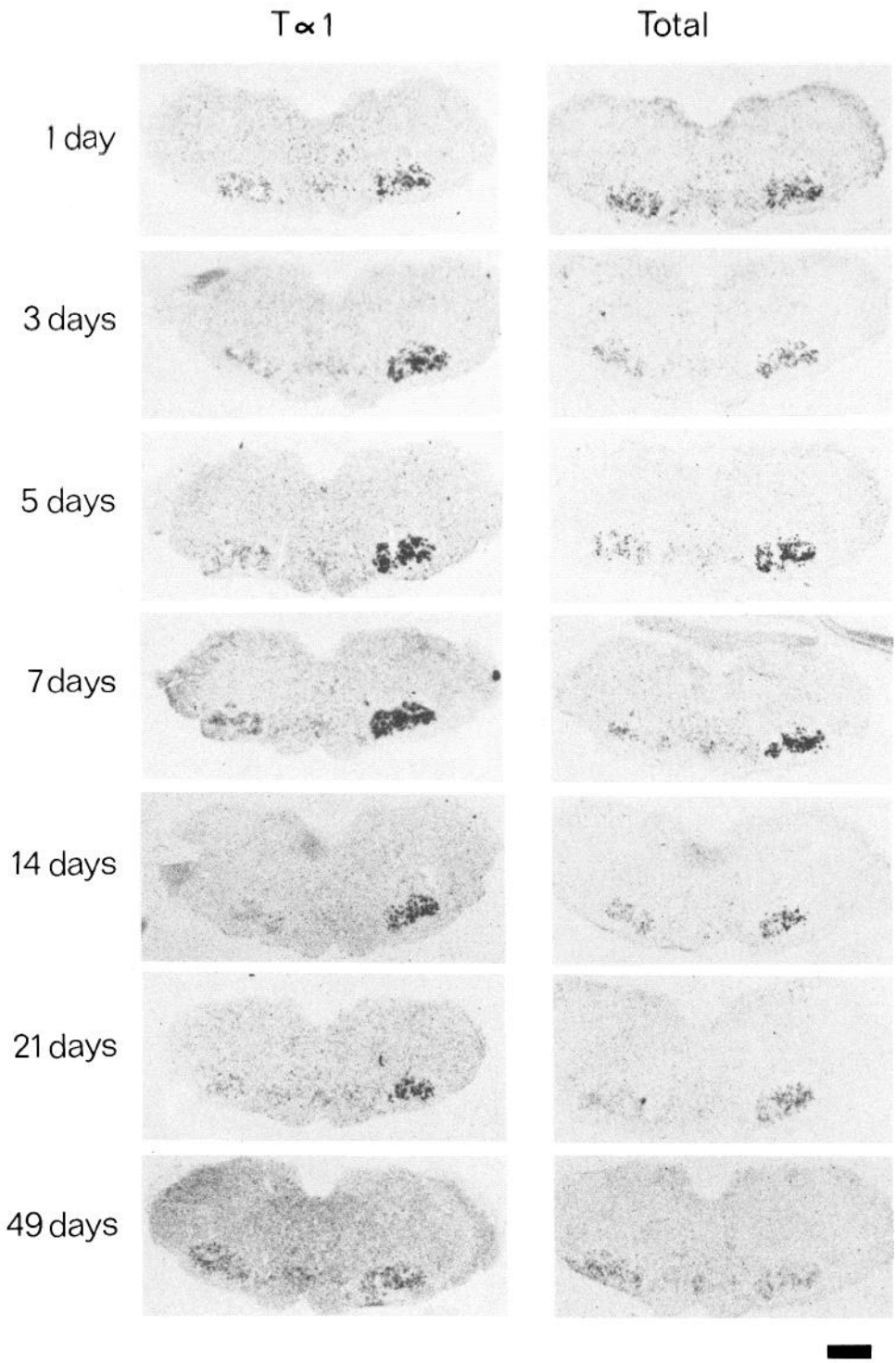


Figure 1. Expression of total and Tα1 α-tubulin mRNAs in control and axotomized facial nuclei following unilateral crush axotomy of the facial nerve. Representative autoradiographs are shown of alternate coronal sections through the facial nuclei of operated rats hybridized to 35S-labeled probes specific to the 3′-untranslated region of Tα1 and the coding region of total α-tubulin mRNA at 1, 3, 5, 7, 14, 21, and 49 d following axotomy. In all photographed sections, the cell bodies of neurons on the operated side are situated to the right of the midline. All sections were counterstained to ensure that both facial nuclei were present within the same section. Scale bar, 1 mm.

system as it is in the developing nervous system, suggesting that the molecular programs underlying axonal growth may be similar in embryonic and adult rats.

An abstract of some of this work has appeared previously (Miller et al., 1987c).

Materials and Methods

Animals and surgical procedures. Male Sprague-Dawley rats weighing approximately 200 g were treated with atropine (1 mg/kg) and anesthetized with ether, after which the facial nerve was transected or crushed unilaterally at the stylomastoid foramen. At least 6 animals were axotomized for each time point of 1 d or less, and at least 2 animals for time points of 3–49 d. Functional reinnervation of facial musculature following axotomy was assayed by the return of vibrissal activity. Following axotomy, animals were sacrificed under deep anesthesia [sodium pentobarbital (32 mg/kg) plus chloral hydrate (800 mg/kg) i.p.]. Facial motor nuclei were punched out of frozen sections of medulla and pooled for biochemical analysis. Alternatively, the animals were anesthetized as above and perfused with 4% paraformaldehyde for *in situ* hybridization analysis.

For sciatic nerve studies, rats were anesthetized with chloral hydrate, and true blue was injected bilaterally under the sciatic nerve epineurium

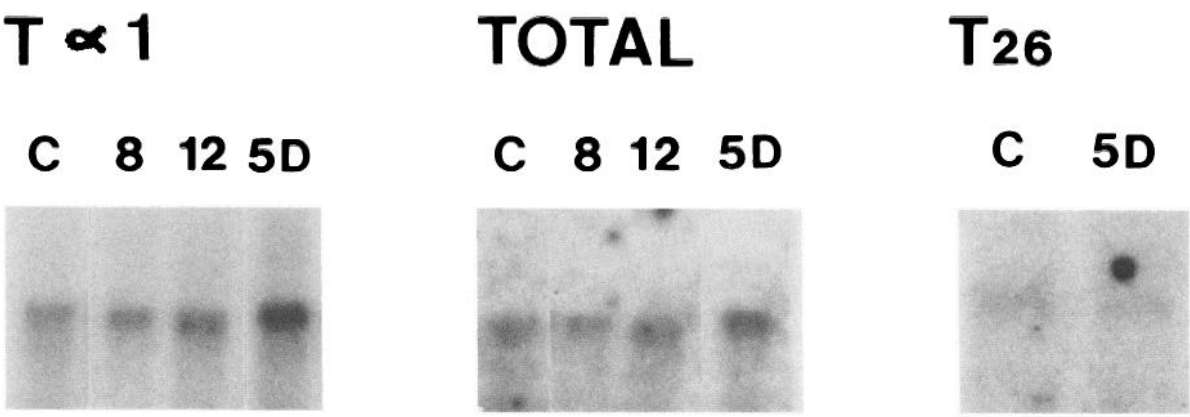


Figure 2. Northern blot analysis of α-tubulin mRNAs in facial nuclei following crush axotomy. At different times after unilateral axotomy, rats were killed and the facial nuclei were punched out of frozen sections of the medulla and pooled. Northern blots of total cellular RNA prepared from control (*C*) facial nuclei or axotomized facial nuclei 8 hr (*8*), 12 hr (*12*), and 5 d (*5D*) postaxotomy were hybridized with 32-labeled probes specific for Tα1 or T26 α-tubulin mRNAs or for the coding region of total α-tubulin mRNA. Rehybridization of the same blots with the clone p1B15 (Milner and Sutcliffe, 1983) demonstrated that similar amounts of RNA were present in each lane and that there was little RNA degradation.

to identify spinal motoneurons. Two weeks later the sciatic nerve was unilaterally crushed or ligated. At different post-operative times, the animals were anesthetized with chloral hydrate and perfused with 4% paraformaldehyde. Spinal cords were removed and processed for *in situ* hybridization analysis. Three axotomized animals were analyzed at each different time point.

Hybridization probes. Probes specific to Tα1, T26, and total α-tubulin mRNAs were prepared as previously described (Miller et al., 1987b). Briefly, a Tα1 α-tubulin cDNA clone (Miller et al., 1987a) was subcloned into pGEM 4 (Promega Biotec), and templates for transcription of riboprobes that were specific for the coding region of α-tubulin mRNA or for the 3′ untranslated region of Tα1 mRNA were prepared by restriction digestion of the subclone with Hind III or Ava II, respectively. Radiolabeled anti-sense RNA probes were generated with SP6 RNA polymerase (Boehringer Mannheim Diagnostics, Houston, TX) and (35S)CTP (New England Nuclear, Boston, MA; 800 Ci/mmol) or 32P-GTP (New England Nuclear, 800 Ci/mmol) under conditions described by the manufacturer and by Melton et al. (1984). To detect T26 mRNA, an oligonucleotide was synthesized that was complementary to part of the unique 3′ coding region of T26 mRNA as previously described (Miller et al., 1987b). Radiolabeled oligonucleotide probes were generated either by end-labeling with (32P) ATP and polynucleotide kinase or by addition of (35S)dATP and terminal transferase, yielding oligonucleotides with approximately 10 labeled residues.

RNA isolation and analysis. Total cytoplasmic RNA was prepared from facial motor nuclei by a modification of the phenol/cloroform/isoamyl alcohol technique (Schibler et al., 1980). RNA samples were fractionated by electrophoresis on 1.2% agarose gels in the presence of 1 M formaldehyde (Rave et al., 1979) and transferred to nitrocellulose (Thomas, 1980). Anti-sense RNA probes labeled with 32P were hybridized to the immobilized RNA as previously described for probes prepared by nick-translation (Lenoir et al., 1986). The 32P end-labeled oligonucleotide probe specific to T26 mRNA was hybridized to the immobilized RNA as described by Woods et al. (1982). Gels were stained with ethidium bromide prior to transfer to nitrocellulose to examine ribosomal RNA for degradation and to confirm that equivalent amounts of samples were loaded in each gel lane.

In situ *hybridization.* Brains from perfused animals were cryoprotected by immersion in graded sucrose solutions and sectioned coronally through the rostrocaudal extent of the facial nuclei. The α-tubulin mRNAs were detected by *in situ* hybridization with 35S-labeled probes specific to the 3′ untranslated regions of Tα1 and T26 and to the coding region of total α-tubulin mRNA, as previously described (Miller et al., 1987b), with the following modifications. Slides were placed in 4% formaldehyde in PBS for 20 minutes, rinsed several times in PBS, and immersed in 50 mM Tris-HCl (pH 7.6) and 5 mM EDTA containing 20 μg/ml proteinase K for 7.5 min. The slides were rinsed in PBS, postfixed in 4% formaldehyde in PBS for 5 min, and dehydrated in graded ethanols containing 0.33 M sodium acetate. Prehybridization solution (1 ml) containing 50% formamide, 0.75 mM NaCl, 25 mM Pipes (pH 6.8), 5× Denhardt's solution (Denhardt, 1966), 0.2% SDS, 100 mM dithiothreitol, 250 μg/ml salmon sperm DNA, and 250 μg/ml yeast tRNA was applied to the slides, which were then incubated for 2–3 hr at 45°C. Coverslips were removed in 4X SSC containing 14 mM 2-mercaptoethanol and washed in 3 rinses of the same solution and in 4 changes of 4× SSC. Slides were subsequently treated with 50 μg/ml RNase A in 0.5 M NaCl, 20 mM Tris-HCl, and 1 mM EDTA for 30 min at 37°C and rinsed in the same buffer for 30 min at 37°C, in 2× SSC for 15 min at room temperature, and in 0.1× SSC for 45 min at 45°C. Slides were air dried and apposed to Dupont Cronex 4 film for 2–48 hr. After the X-ray film images were analyzed, the slides were dipped in Kodak NTB-2 emulsion, exposed for 12 hr to 7 d (depending on the intensity of the X-ray image), developed, counterstained with hematoxylin and eosin, and analyzed by brightfield and darkfield microscopy. Controls were done to ensure specificity of hybridization, including prehybridizing or cohybridizing slides with a 100-fold excess of cold anti-sense RNA in addition to 35S-labeled riboprobe or with a labeled RNA probe transcribed in the sense direction.

Analysis and quantification. To verify that the *in situ* hybridization results were quantitative and reproducible, we routinely analyzed grain densities in facial motor neurons or sciatic motor neurons in the spinal cord of unoperated animals. Motoneurons on opposite sides of the same section or on adjacent sections on the same slide reproducibly had similar grain densities. To verify qualitative differences observed on X-ray film, emulsion-dipped slides were counterstained with hematoxylin and eosin, and grains were counted in double-labeled motor neurons.

Results

The developmental pattern of α-tubulin gene expression is specifically reinduced in regenerating facial motoneurons

To define the pattern of α-tubulin gene expression during regeneration, facial motoneurons of adult rats were unilaterally crushed and coronal sections through the facial nuclei were hybridized to probes specific to the 3′ untranslated regions of Tα1 or T26 α-tubulin mRNAs or to a coding region probe for total α-tubulin mRNA. The hybridization signal for Tα1 mRNA was elevated in the axotomized facial nucleus 24 hr following axotomy (Fig. 1). The intensity of the Tα1 signal in the operated facial nucleus increased relative to the control nucleus to a peak between 3 and 7 d following axotomy. Levels of Tα1 mRNA subsequently started to decline slowly between 14 and 21 d following axotomy (reinnervation of facial muscles is established between 10 and 15 d postcrush, as assayed by the return

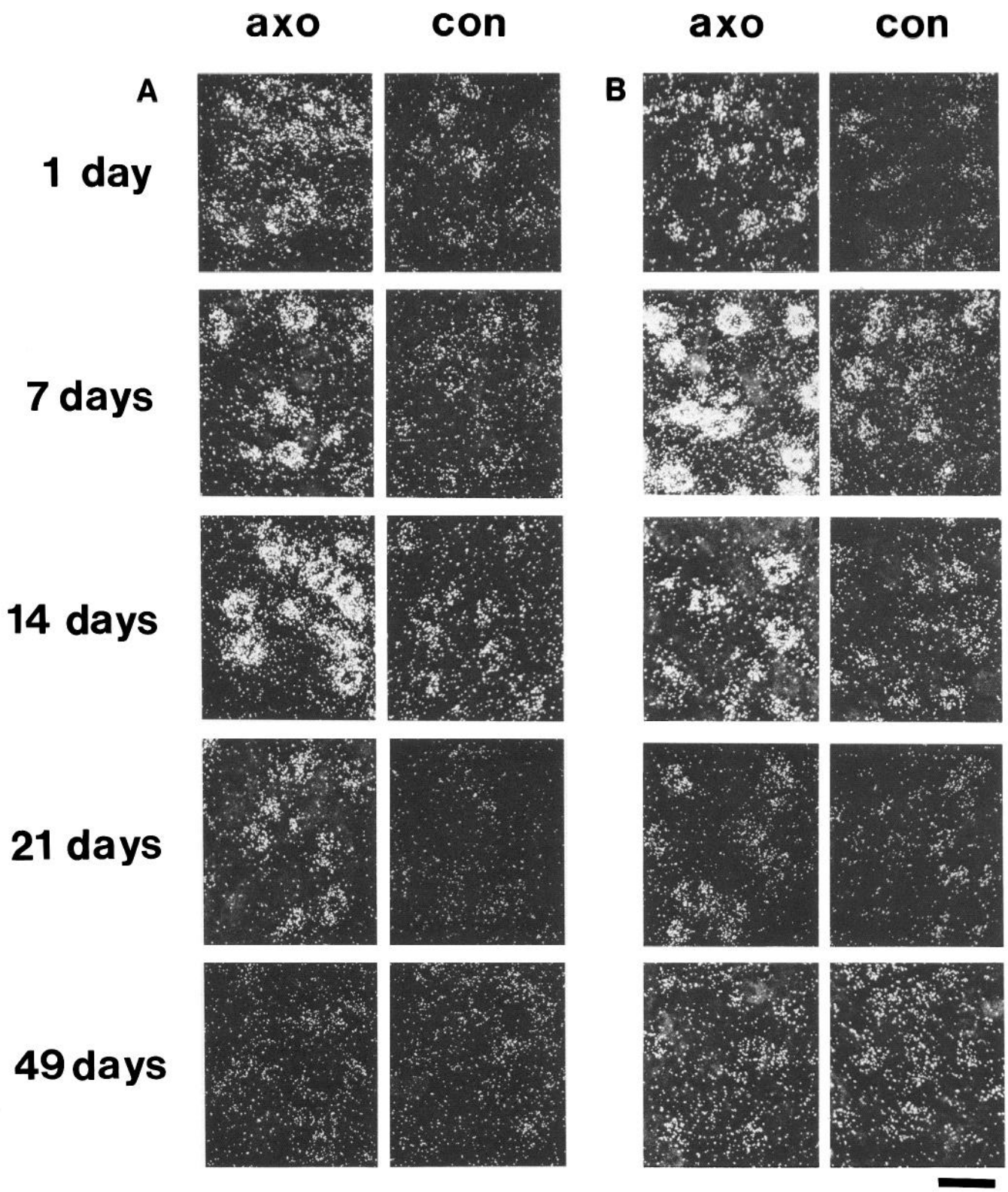
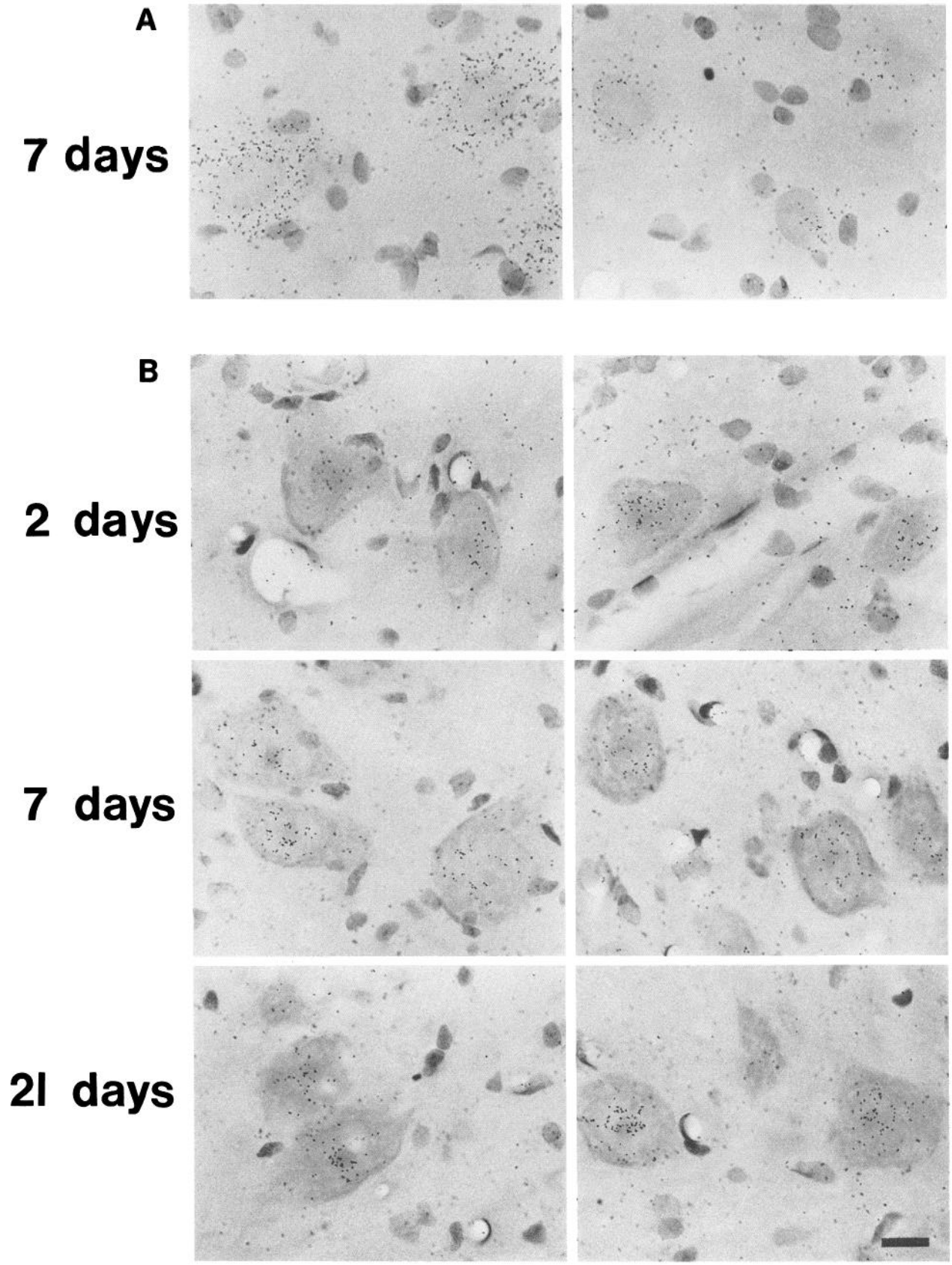


Figure 3. Expression of α-tubulin mRNAs in facial motor neurons following crush axotomy. Sections through axotomized (*axo*) and control (*con*) facial nuclei at 1, 7, 14, 21, and 49 d after axotomy were hybridized with probes specific for Tα1 (*A*) or total (*B*) α-tubulin mRNA. Hybridized sections were coated with emulsion for autoradiography, developed, counterstained, and photographed under darkfield illumination. All photographs were exposed and developed under identical conditions. The axotomized and control neurons were photographed from the same section. Scale bar, 20 μm.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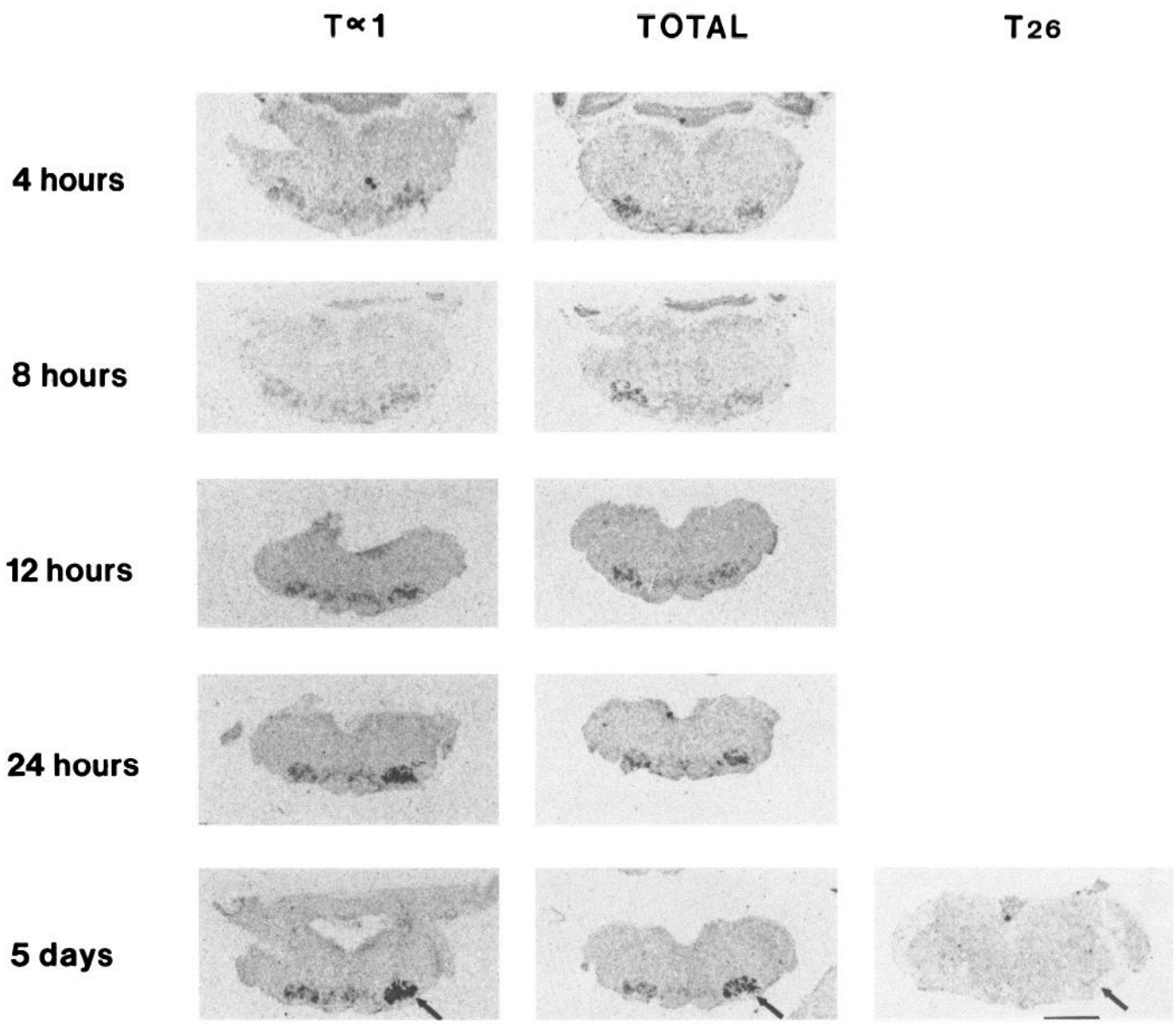

Figure 5. Expression of α-tubulin mRNAs in control and axotomized facial nuclei at early time points following unilateral crush axotomy of the facial nerve. Representative autoradiographs are shown of alternate coronal sections through the facial nuclei of operated rats hybridized to 35S-labeled probes specific to the 3′-untranslated region of Tα1 mRNA and the coding region of total α-tubulin mRNA at 4, 8, 12, and 24 hr, and 5 d, or a probe specific to the 3′-untranslated region of T26 α-tubulin mRNA at 5 d following axotomy. In all sections, the cell bodies corresponding to the axotomized nerve are present to the right of the midline (*arrows*). Scale bar, 2 mm.

of vibrissal activity). Seven weeks postaxotomy, the Tα1 hybridization signal was similar in control and operated nuclei.

Induction of Tα1 α-tubulin mRNA was confirmed by Northern blot analysis of mRNA isolated from facial nuclei punches (Fig. 2). Increased Tα1 mRNA in operated nuclei was reproducibly detected 8 hr after axotomy and continued to increase through 5 d. The time course demonstrated by Northern blot analysis is consistent with that determined by *in situ* hybridization.

To determine whether changes in Tα1 mRNA occurred in neurons or glial cells (Tetzlaff et al., 1988b), we examined hybridized sections at higher resolution (Figs. 3, 4). In control animals, Tα1 mRNA expression is generally low, although it is increased in neurons that maintain long axonal projections or

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Figure 4. Expression of Tα1 and T26 α-tubulin mRNAs in facial motor neurons following crush axotomy. Sections through axotomized (*AXO*) and control (*CON*) facial nuclei at 7 d (*A*) or at 2, 7, and 21 d (*B*) after axotomy were hybridized with probes specific for Tα1 (*A*) or T26 (*B*) α-tubulin mRNA, respectively. Hybridized sections were coated with emulsion for autoradiography, developed, counterstained with hematoxylin and eosin (Tα1) or with cresyl violet (T26), and photographed under brightfield illumination. For Tα1 mRNA, the grains are localized around the large, pale-staining neuronal nuclei. For T26 mRNA, there is no change in the number of grains localized over the large facial motoneurons at any of the time points examined. Scale bar, 5 μm.

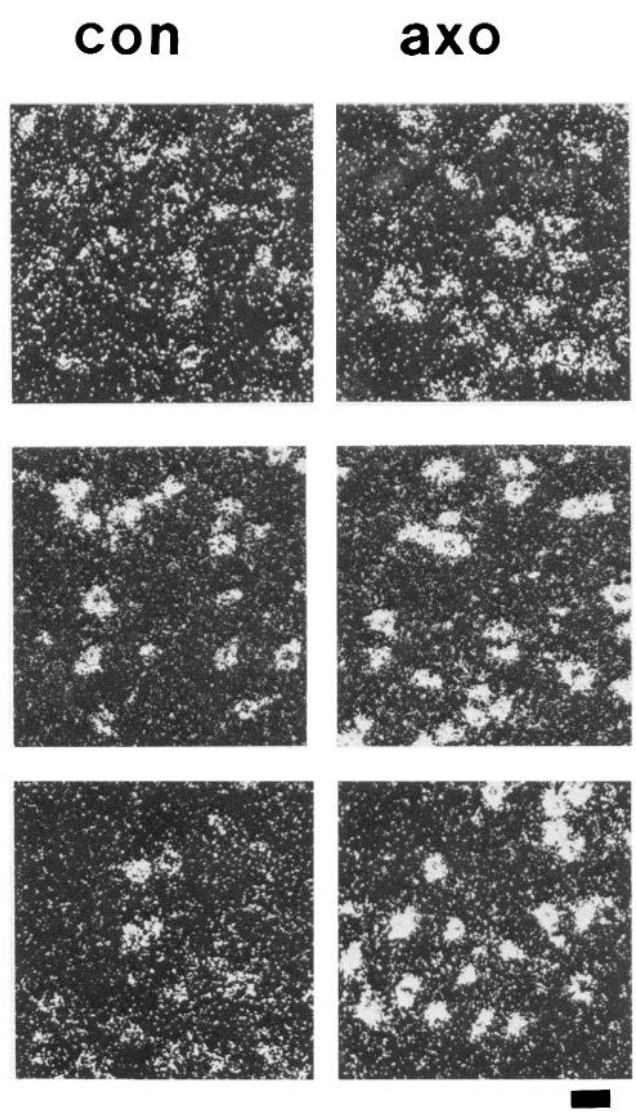

Figure 6. Expression of Tα1 α-tubulin mRNA in facial motor neurons at early time points following crush axotomy. Sections through axotomized (*axo*) and control (*con*) facial nuclei at 4 hr, 8 hr, and 5 d after axotomy were hybridized with probes specific for Tα1 α-tubulin mRNA. Hybridized sections were coated with emulsion for autoradiography, developed, counterstained, and photographed under darkfield illumination. Scale bar, 20 μm.

elaborate dendritic trees, including those of the facial nucleus. The intensity of this signal increased (as measured by grain counting on emulsion-dipped sections) at 24 hr and peaked 7–14 d after axotomy (Fig. 3); the increased grains were consistently localized to the axotomized motoneurons (Fig. 4). Subsequent changes in Tα1 mRNA from 14–49 d, as detected on the autoradiographs, could also be attributed to changes in facial motoneuron gene expression (Fig. 3).

In contrast to Tα1 α-tubulin mRNA, levels of mRNA for T26 α-tubulin in facial nuclei did not change following axotomy. In control animals, T26 mRNA is expressed at low, constitutive levels in glial cells and in neurons (Durand et al., unpublished observations). *In situ* hybridization analysis revealed that the level of expression of T26 mRNA in facial motoneurons was low and remained unchanged at 1, 2, 3, 5, 7, 14, 21, and 49 d following crush of the facial nerve (representative timepoints are shown in Figs. 4, 5). Northern blot analysis confirmed that the abundance of T26 mRNA in facial nuclei was not increased at 5 d postaxotomy, a time when Tα1 α-tubulin mRNA levels were at their peak in the operated motoneurons (Fig. 2).

We also examined changes in the expression of total α-tubulin mRNA. RNase-protection analysis done on control facial nuclei revealed that Tα1 mRNA comprises a relatively small fraction of the total α-tubulin mRNA (data not shown). In contrast to Tα1 mRNA, levels of total α-tubulin mRNA showed only a slight increase at 1 d postaxotomy, as revealed by *in situ* hybridization (Fig. 1). Levels of total α-tubulin mRNA peaked at the same time as Tα1 mRNA, although the relative increase

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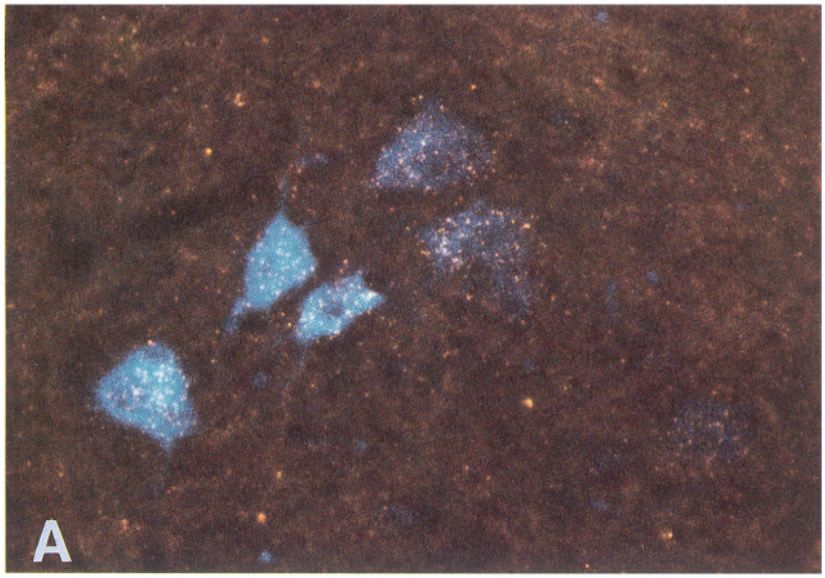
Figure 7. Expression of Tα1 α-tubulin mRNA in the cell bodies of motor neurons contributing to the sciatic nerve. Horizontal sections were taken through the appropriate level of the spinal cord of perfused, operated rats, and hybridized to a 35S-labeled probe specific to the 3′-untranslated region of Tα1 mRNA. Hybridized sections were coated with emulsion for autoradiography, developed, and photographed under fluorescence (*B*), darkfield (*C*), or combined fluorescence/darkfield (*A*) illumination. Double-labeled motor neurons were compared on the control (*D*) and operated (*E*) sides of the same sections for qualitative and quantitative analyses. In the case shown, the animal was killed 7 d postcrush. Calibration bar, 5 μm.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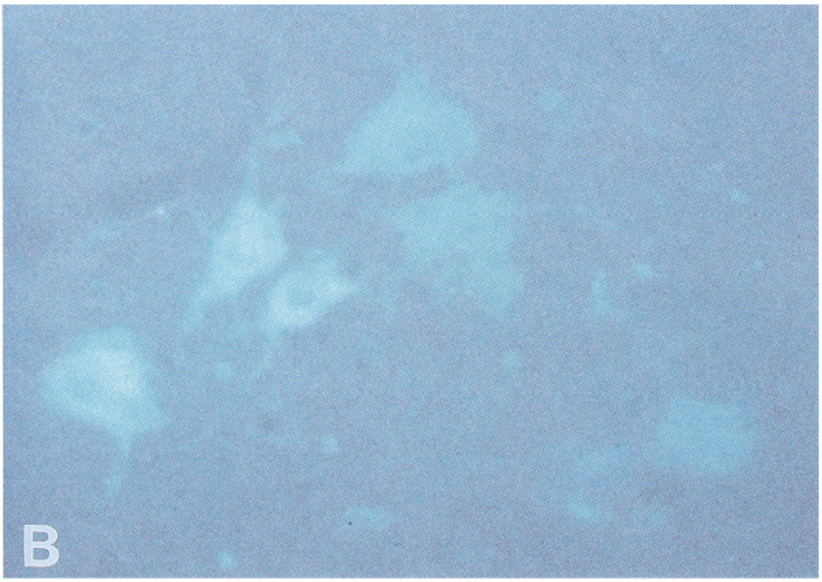
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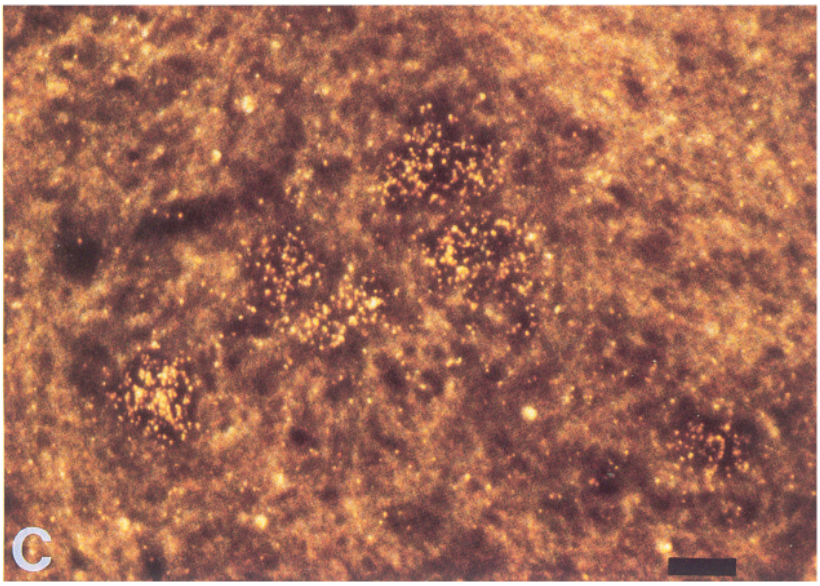
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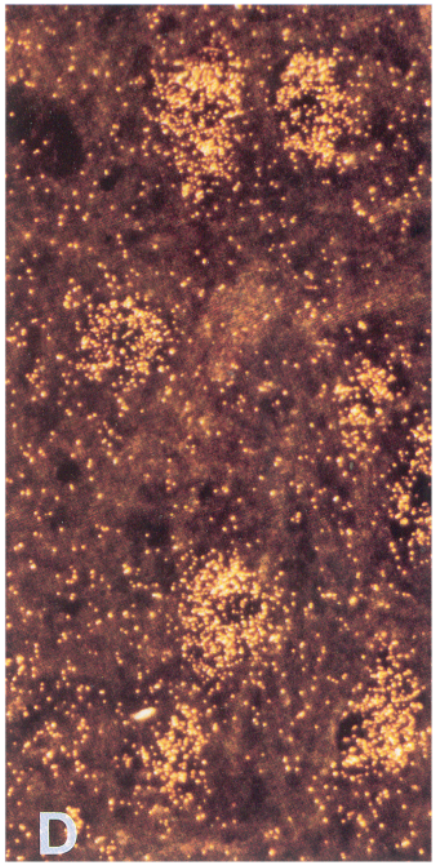
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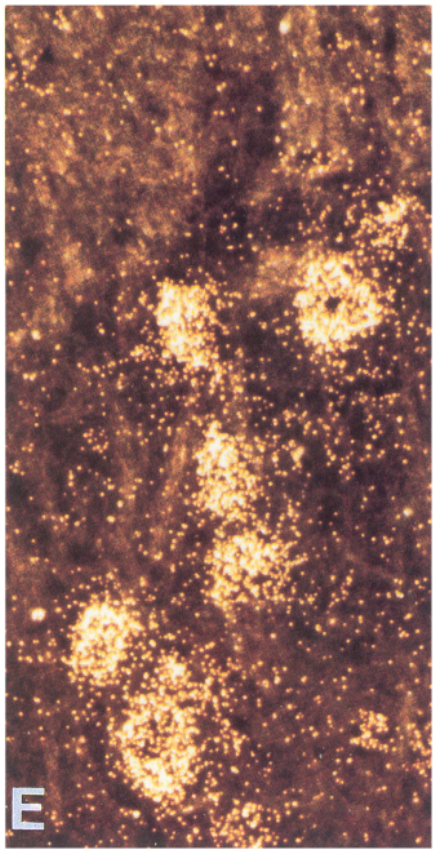
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was not as great, as determined by Northern blot analysis (Fig. 2). The amount of total α-tubulin mRNA began declining at 2 weeks following the crush and reached control levels after 7 weeks (Fig. 1). Microscopic examination of the dipped tissue sections confirmed the time course at the cellular level and demonstrated that changes in total α-tubulin mRNA levels occurred solely in neurons (Fig. 3).

The major embryonic α-tubulin mRNA is reinduced within 4 hr of axonal damage

By 24 hr postlesion, levels of Tα1 α-tubulin mRNA were significantly higher in the operated than in the control facial motoneurons (see above). To determine how quickly the response occurred, we measured Tα1 α-tubulin mRNA in nuclei from control and injured nerves at 2, 4, 6, 8, and 12 hr postcrush. The hybridization signal for Tα1 mRNA was reproducibly elevated at 4 hr following axotomy in 6 different rats (Fig. 5); grain-counting on the emulsion-dipped sections (Fig. 6) revealed that the amount of Tα1 mRNA was approximately 1.5-fold higher in operated than in control neurons at this time point. By 12 hr postcrush, the difference between control and injured motor neurons had increased to approximately 2-fold.

In contrast, the amount of total α-tubulin mRNA was similar in control and operated facial nuclei over the same time course, and, in most animals, was actually lower on the operated side (Fig. 5).

Tα1 α-tubulin mRNA is induced in regenerating sciatic motor neurons

To determine whether the induction of Tα1 α-tubulin mRNA was specific to damaged facial motor neurons or was part of a more generalized neuronal response, we examined its expression in spinal cord motoneurons following crush of the sciatic nerve. True blue was injected bilaterally into the sciatic nerve sheath 2 weeks prior to unilateral nerve crush so that motor neurons distributed within the sciatic nerve could be identified in horizontal sections of the spinal cord (Fig. 7, *A–C*). Animals were killed at selected time points 1–15 d postaxotomy. *In situ* hybridization analysis for Tα1 α-tubulin mRNA revealed that Tα1 mRNA was slightly higher in the operated than control motor neurons 1 d following nerve crush (Fig. 8*B*). The relative amount of Tα1 mRNA in the operated neurons increased to a peak between 3 and 7 d postaxotomy (Fig. 7, *D, E*; Fig. 8*B*) and was maintained at the same level 15 d postcrush.

We also examined the expression of total α-tubulin mRNA (data not shown). As in the facial motoneurons, the general time course of total α-tubulin mRNA expression following axotomy was similar to that for Tα1 mRNA. However, the relative increase in total α-tubulin mRNA was consistently lower than that of Tα1 mRNA.

Expression of Tα1 α-tubulin mRNA can be modulated during regenerative axonal growth

As a step towards defining the factors that regulate Tα1 α-tubulin mRNA expression during regeneration, we compared its expression in the motor neurons of crushed vs. tied sciatic nerves. Ligature of the nerve, which impedes regeneration of axons into the distal nerve and target reinnervation, also led to reinduction of Tα1 α-tubulin mRNA. There was a small increase in Tα1 mRNA in the ligated motor neurons by 1 d postaxotomy (Fig. 8*A*). Levels of Tα1 mRNA increased in the axotomized motoneurons to a peak at 7 d and remained elevated at 15 d following ligation. The initial increase and subsequent peak in Tα1 mRNA expression in the ligated motoneurons were similar to those detected in crushed motoneurons. Tα1 mRNA levels were approximately 2–3-fold higher in the ligated or crushed versus control motoneurons at 7 d postaxotomy, as detected by grain-counting on emulsion-dipped sections (Fig. 8, *A, B*).

In order to extend these observations, we examined the expression of Tα1 α-tubulin mRNA in crushed versus transected facial motor neurons. At 1, 3, 5, 7, and 14 d postaxotomy, the relative increase in levels of Tα1 α-tubulin mRNA were similar in crushed (Fig. 3) and transected facial nuclei (data not shown). Between 14 and 21 d postaxotomy, the abundance of Tα1 mRNA in the transected motoneurons declined (Fig. 9), as it did for the crushed motoneurons (Fig. 3). However, at 49 d following injury, when Tα1 mRNA levels were back to control levels in the crushed neurons (Fig. 3), the amount of Tα1 mRNA was still elevated in transected neurons (Fig. 9).

Discussion

In this paper we report that 2 rat α-tubulin isotypes, Tα1 and T26, are differentially regulated during regeneration of adult rat motor neurons. Tα1 α-tubulin mRNA, which is expressed at high levels during developmental neurite outgrowth, is induced following axotomy. In contrast, expression of T26 α-tubulin mRNA, which is not regulated during neural development, does not change during the regenerative process. The induction of Tα1 mRNA therefore appears to be a specific, growth-associated response of motor neurons to axonal injury. It is likely that the growth response is multigenic in nature. We have observed that Devo 8 mRNA (Miller et al., 1987a), a polyadenylated mRNA that encodes the histone H3.3 variant (Wells and Kedes, 1985) and is enriched during neuronal development, is also induced during regeneration of facial motoneurons (unpublished observations). Further, a recent report demonstrates that a class II β-tubulin mRNA is specifically reinduced in regenerating sensory neurons of the sciatic nerve (Hoffman and Cleveland, 1988). In contrast, the abundance of the mRNAs for the neurofilament proteins NF68 and NF140, molecules that are associated with mature neurons, declines during the initial phases of neuronal regeneration in facial motoneurons (Tetzlaff et al., 1987) and, for NF68, in sensory neurons of the sciatic nerve (Hoffman et al., 1987; Wong and Oblinger, 1987). The decrease in total α-tubulin mRNA at earlier time points that was detected in most of the experimental animals may be explained by invoking a similar decrease in the abundance of α-tubulin mRNAs that are expressed in the mature motoneurons. Thus, our results with facial nerve axotomy suggest that during regeneration a rapid "switch" occurs in these neurons, from a program designed to maintain cell structure to a growth-associated pattern of gene expression.

The induction of Tα1 α-tubulin mRNA is not specific to regenerating facial motor neurons; since it occurs also in motor neurons of the sciatic nerve. More recent data demonstrate that Tα1 α-tubulin mRNA is also reinduced in mature axotomized sympathetic neurons of the superior cervical ganglion (Mathew et al., 1988), suggesting that induction of Tα1 mRNA may occur during process extension in both the peripheral and the central nervous system. In support of this idea, it has been demonstrated that Tα1 α-tubulin mRNA is reinduced in uninjured hippocampal neurons following a partial entorhinal cortical lesion (Geddes et al., 1988).

Tα1 α-tubulin mRNA was induced very rapidly in all of these

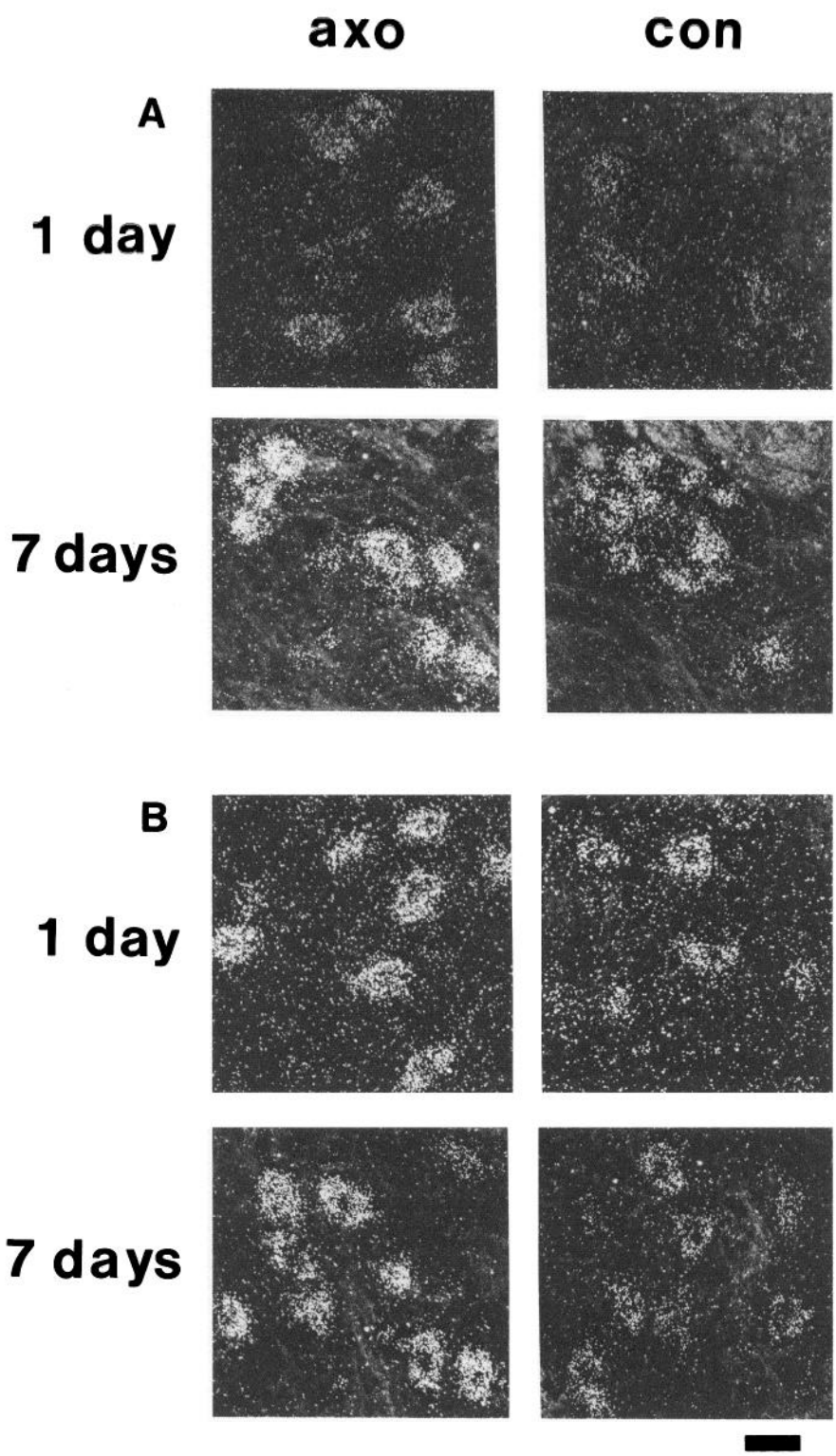

Figure 8. Expression of Tα1 α-tubulin mRNA in crushed versus ligated motor neurons. Sections through axotomized (*axo*) and control (*con*) sciatic motor neurons from ligated (*A*) versus crushed (*B*) nerves at 1 d and 7 d after axotomy were hybridized with a probe specific for Tα1-tubulin mRNA. Hybridized sections were coated with emulsion for autoradiography, developed, and photographed under darkfield illumination. The axotomized and control neurons were photographed from the same section. Scale bar, 10 μm.

experimental studies. In axotomized facial motoneurons, the neuronal cell body, which is 1.5 cm from the site of axonal injury, responds to axonal damage with enhanced gene expression within 4 hr. The cellular mechanisms involved in activating this rapid response are unknown, but could involve retrograde transport of signals from the site of injury, depletion of retrograde trophic factors derived from target tissues, or other as yet undefined signals.

Following the initial injury-induced reinduction of the Tα1 α-tubulin gene, levels of the mRNA rise slowly to a plateau by 3 d postcrush and remain elevated during the entire period of axon regrowth. Two possibilities could account for these results. Subsequent to induction, Tα1 α-tubulin mRNA could be expressed at a steady level until it is down-regulated by an environmental factor such as the outgrowing axon encountering the appropriate target. Alternatively, maintenance of Tα1 mRNA

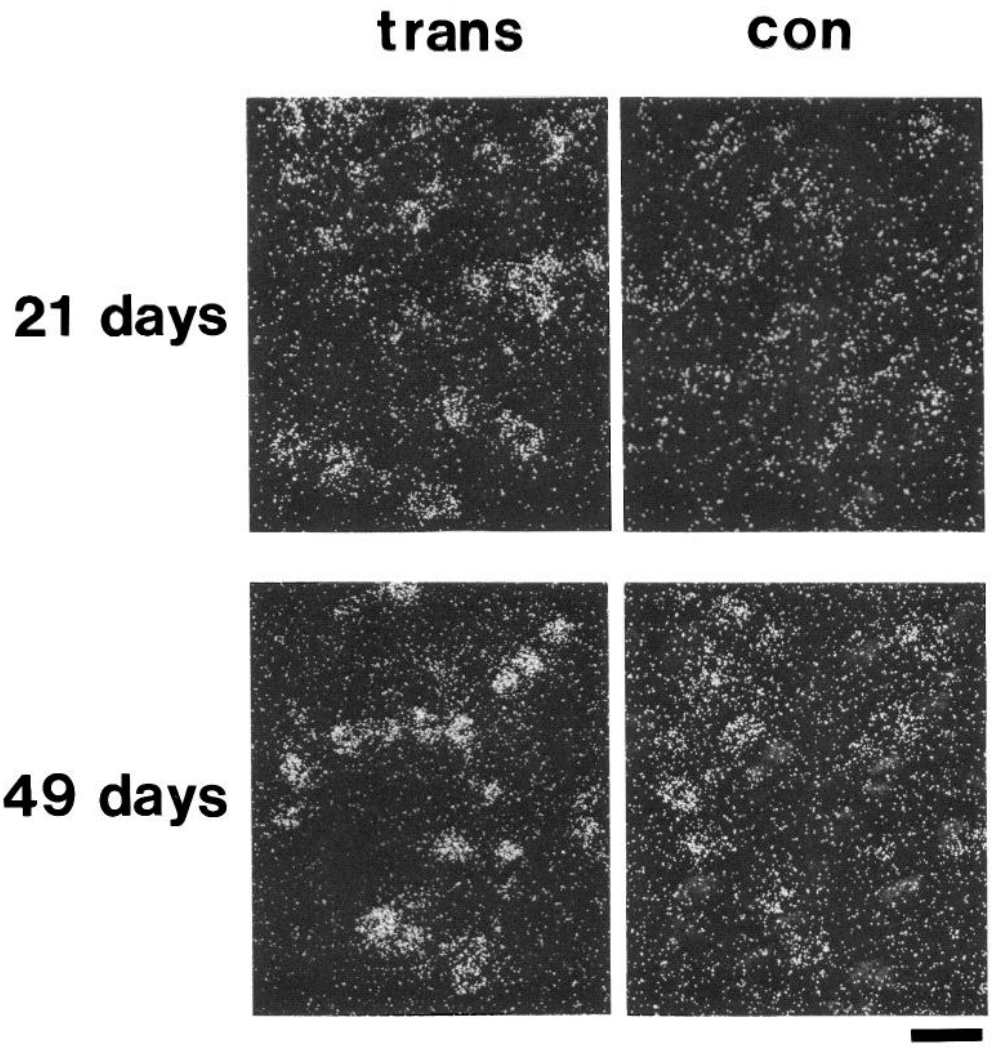

Figure 9. Expression of Tα1 α-tubulin in transected facial motor neurons. Sections through axotomized (*axo*) and control (*con*) facial motor nuclei at 21 and 49 d after axotomy were hybridized with a probe specific for Tα1 mRNA. Hybridized sections were coated with emulsion for autoradiography, developed, and photographed under dark-field illumination. The axotomized and control neurons were photographed from the same section. Scale bar, 20 μm.

levels may require interaction between the axon or its cell body and other cell types or extracellular components during the regenerative period. This question was addressed by the experiment involving ligature vs. crush of the sciatic nerve. From 1–15 d postaxotomy, Tα1 α-tubulin mRNA levels are similar in tied and crushed motor neurons. These results indicate that contact between the growing axons and the basal lamina of the denervated nerve sheath is not necessary for maintenance of Tα1 mRNA expression. Tα1 mRNA expression may, however, be maintained by contact with or production of factors by Schwann cells or fibroblasts in the immediate vicinity of the ligature. For example, Schwann cells respond to axotomy by expressing both NGF mRNA (Heumann et al., 1987) and NGF receptor protein (Taniuchi et al., 1988). Although motor neurons are not responsive to NGF, Schwann-cell-derived trophic support of some other nature may be important for maintenance of the growth-associated neuronal phenotype.

The Tα1 α-tubulin gene remains active in facial motor neurons until the approximate time of functional reinnervation (10–15 d postaxotomy), when the gene is subsequently down-regulated. The time of down-regulation may be programmed genetically (i.e., at 7–14 d after induction, the gene will turn off), or it may be a function of target:neuron interactions which serve to "switch" the neurons back from a growth-associated to a mature neuronal phenotype. Because Tα1 α-tubulin mRNA levels remain elevated longer in the transected than in the crushed facial motor neurons, as demonstrated here, it is likely that target:neuron interactions are at least partially responsible for turning off Tα1 mRNA expression. It is possible that Tα1 α-tubulin mRNA remains up-regulated for long periods in neurons when specific target tissues are removed or when the axon is physically prevented from innervating the appropriate target.

In summary, the major embryonic α-tubulin mRNA, Tα1, is specifically and rapidly reinduced following axotomy, is maintained at steady levels during axonal growth, and is subsequently down-regulated when axons reinnervate their targets, in a manner reminiscent of neuronal development. These data therefore support, at a genetic level, the long-standing hypothesis that successful neuronal regeneration recapitulates neuronal development (Van Biervliet, 1900; Brattgard et al., 1957; Griffith and La Velle, 1971). Axotomy rapidly induces a developmental pattern of neuronal gene expression that subsequently may be regulated by environmental factors such as axon:Schwann cell and axon:target cell interactions. It will be of interest to know which

environmental signals induce expression of Tα1 α-tubulin and maintain or modulate its expression during regenerative axonal growth.

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