lysine (Sigma). After 48 hours, cells were washed three times with defined culture medium. Factors were added to dishes that contained 1.5 ml of defined medium.
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14. IL-1 was separated rapidly from the AMoL cell culture medium by hollow-fiber and membrane ultrafiltration. Culture medium (8 liters) was thawed and gravity-filtered through Whatman paper to remove debris. The sample was concentrated to 1 liter by means of a hollow-fiber device (50,000 molecular weight cutoff; Amicon), and both the ultrafiltrate and retained material were saved. The retained material was diafiltered by means of the same hollow fibers with 10 liters of 0.85 percent NaCl, and the diafiltrate and previous ultrafiltrate were pooled (17 liters). The sample was ultrafiltered with a 2-liter stirred cell (Amicon) containing a YM30 membrane (30,000 molecular weight cutoff). The ultrafiltrate (~17 liters) was retained and concentrated to 40 ml with the same stirred cell containing a YM10 membrane (10,000 molecular weight cutoff).
15. For HPLC, a 10-ml sample was dialyzed against water for 3 days and lyophilized. The sample was hydrated in 2.0 ml of 0.02*M* tris–acetic acid (*p*H 8.0, buffer A) and applied to a TSK DEAE–5-PW column (75 by 7.5 mm; Bio-Rad) at a flow rate of 1.0 ml/min. The column was developed with the indicated gradient of buffer A containing 0.5*M* sodium acetate (Fig. 3). Individual fractions were assayed by the thymocyte assay at 0.001 percent (by volume). Sucrose-gradient isoelectric focusing of a 5-ml sample of the 10- to 30-kD fraction was performed in a *p*H 4 to 8 gradient as described (*16*). Individual fractions were dialyzed for 72 hours against 0.85 percent NaCl before the thymocyte or glial cell assays.
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# A Specific 37,000-Dalton Protein That Accumulates in Regenerating but Not in Nonregenerating Mammalian Nerves

Abstract. *A 37-kilodalton protein is synthesized at higher rates in the peripheral and central nervous system of newborn rats than in adult animals. As a specific response to denervation, the synthesis of the 37-kilodalton protein is increased in the mature peripheral and central nervous system; however, this protein accumulates only in the peripheral nervous system. The differences in accumulation of the protein correlate with the apparent differences in the ability of peripheral and central axons to regenerate. The synthesis of the 37-kilodalton protein is inhibited when proper innervation or reinnervation is established.*

Axons in the peripheral nervous system (PNS) of mammals can regenerate after injury, whereas axons in the adult mammalian central nervous system (CNS) typically show only abortive sprouting after lesions (*1–3*). Experiments showing extensive elongation of central axons into peripheral nerve grafts (*4*) suggested that the microenvironment of regrowing axons plays a decisive role in the success or failure of regeneration. Additional evidence supporting this hypothesis was obtained from experiments showing that PNS axons, which are known to have a capacity to regenerate, did not elongate into an environment of CNS glia (*3, 5*). Molecular interactions between axons and their adjacent glial

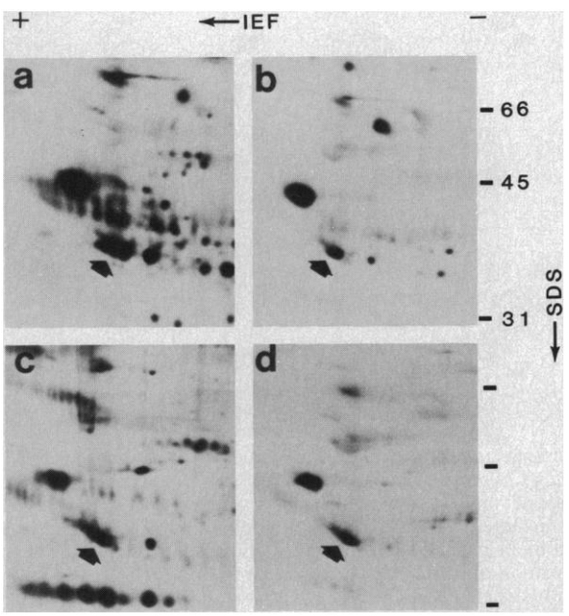
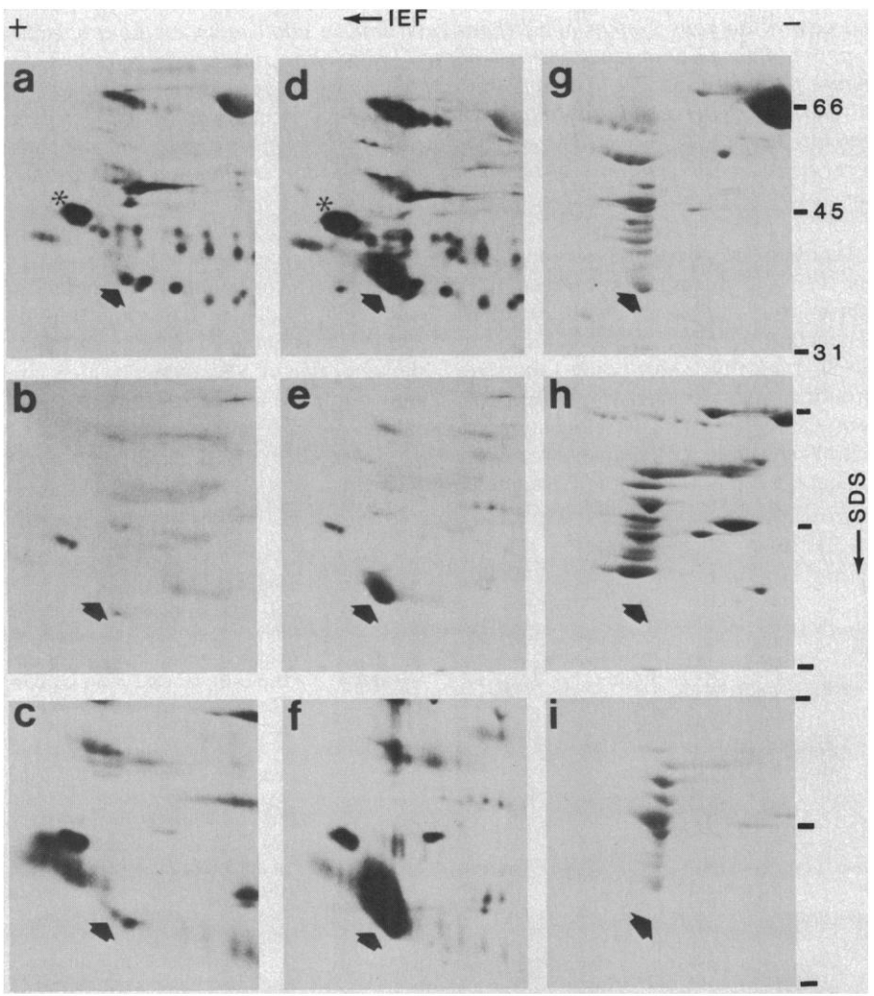



Fig. 1. Synthesis and secretion of the 37-kD protein in the developing PNS and CNS of newborn rats. Analyses of isotopically labeled proteins corresponding to (a) four sciatic nerves (wet weight of tissue, ~7 mg by weight), (b) a 0.5-cm segment of spinal cord (12 mg by weight), (c) eight optic nerves (4 mg by weight), and (d) a portion of cerebral hemisphere (20 mg by weight) are shown. The dissected nervous tissue was chopped and incubated with 100 μCi of [$^{35}$S]methionine in Dulbecco's modified Eagle's medium for 4 hours at 37°C. Extracellular soluble and secreted proteins released by the nerves were collected in the incubation medium, which was then dialyzed against 50 m*M* ammonium acetate before lyophilization. The dried protein samples were analyzed by 2D PAGE as described (*7*). The first dimension was isoelectric focusing (IEF) in 4.2 percent polyacrylamide gels containing 4 percent ampholytes at *p*H 3.5 to 10 and 2 percent ampholytes at *p*H 4 to 6 (LKB-Produkter AB). The second dimension was carried out in 10 percent polyacrylamide gels containing sodium dodecyl sulfate under reducing conditions (*12*). Gels were stained with Coomassie brilliant blue before autoradiographic image enhancement with EN$^3$HANCE (New England Nuclear) and then dried and exposed to presensitized Kodak XAR-5 film. The amount of protein in a, b, and c was approximately 50 μg and in d was 5 to 10 μg. The arrows indicate the position of the 37-kD protein. The numbers to the right indicate molecular mass standards (Bio-Rad): bovine serum albumin (66 kD), ovalbumin (45 kD), and carbonic anhydrase (31 kD).

cells are likely to play an important role in effective neuronal regeneration after axonal injury (*6*). Recently, Skene and Shooter (*7*) described the increased synthesis and secretion of a 37-kilodalton (kD) protein in the distal segment of crushed rat sciatic nerve. The increased synthesis of a homologous 37-kD protein after injury in the CNS has also been shown in rat optic nerve (*7, 8*). It is of interest to determine whether or not the change in the expression of this protein is a result of altered relations between axons and glia.

We now describe the expression of the 37-kD protein during de novo nerve growth in the PNS and CNS of newborn rats, the specific differences in post-traumatic expression of this protein in adult rat PNS and CNS, and the inhibition of synthesis of the protein apparently by a signal or signals from regenerating axons.

To investigate the expression of the 37-kD protein during de novo nerve growth in the PNS and CNS, we dissected sciatic nerve, spinal cord, optic nerve, and brain from 1-week-old Sprague-Dawley rats. Proteins in the isolated nerve tissue were metabolically labeled by incubation with [$^{35}$S]methionine as described (*7*). Extracellular soluble and secreted proteins released by the nerves into the incubation medium were separated by two-dimensional polyacrylamide gel electrophoresis (2D PAGE) as described (*9*). Isotopically labeled proteins were subsequently identified by fluorography. The 37-kD protein was synthesized in both the developing PNS and CNS at similar rates (Fig. 1). These rates were increased compared to those



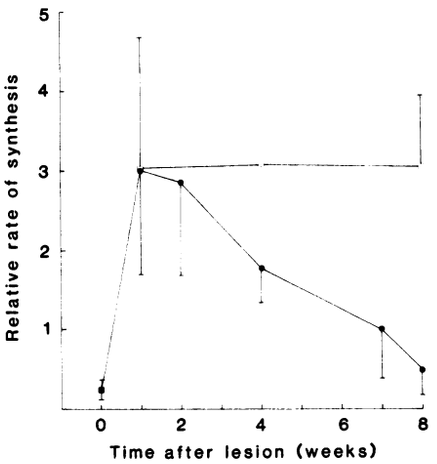




Fig. 2 (left). Comparison of synthesis and accumulation of the 37-kD protein in denervated PNS and CNS of adult rats. Sprague-Dawley rats weighing 180 to 200 g were anesthetized with sodium pentobarbital (4 to 6 mg per 100 g of body weight) and chloral hydrate (10 mg per 100 g of body weight) administered intraperitoneally. Sciatic nerves were crushed with jeweler's forceps at the upper thigh, and optic nerves were crushed in the orbit as described (*7*). Hemisection of spinal cord was carried out with a pair of scissors between the last thoracic and the first lumbar segment after laminectomy. The neural tissue was removed 10 to 14 days after surgery, chopped into 1- to 2-mm segments, and incubated with [$^{35}$S]methionine, and the labeled proteins released into the medium were analyzed by 2D PAGE as described in the legend of Fig. 1. (a to c) Fluorographs of labeled proteins from undamaged (control) sciatic nerve (a), spinal cord (b), and optic nerve (c) of sham-operated animals. (d to f) Fluorographs of labeled proteins from the distal segments of a crushed sciatic nerve (d), the thoracic region of a hemisected spinal cord (e), and the distal stump of a crushed optic nerve (f). (g to i) Corresponding Coomassie-blue staining of the gels represented by d, e, and f. The amounts of protein loaded on the gels were approximately 100 μg for sciatic nerve and spinal cord and approximately 50 μg for optic nerve. For the optic nerve, the incubation medium of eight crushed nerves was pooled to increase the amount of protein for analysis. The arrows indicate the position of the 37-kD protein. The asterisks in (a) and (d) mark a 43-kD protein in sciatic nerve medium whose relative labeling did not change upon injury. The numbers and marks to the right of the gels indicate the position of molecular mass standards (see Fig. 1). Fig. 3 (right). Relative rates of synthesis of the 37-kD protein in uninjured (■), permanently denervated (□), and regenerating (●) rat sciatic nerves. Vertical bars represent the standard deviation of four to six experiments with individual nerves. The value estimated for the 4-week time point in the permanently denervated nerve represents the mean of two independent experiments. In one series of experiments, the left sciatic nerves of 13 adult rats were exposed, and two double ligations with 4-0 suture thread were applied in close proximity on the nerve at the upper thigh. The nerves were then transected with a pair of fine scissors between the ligations, and both stumps were oriented away from each other and sewed into local muscle tissue. In another series of experiments, the left sciatic nerves of 15 rats were crushed with jeweler's forceps. The sciatic nerves of four sham-operated animals were used as controls. Proteins in the nerve segments were metabolically labeled by incubation with [$^{35}$S]methionine, and the labeled proteins released into the medium were analyzed by 2D PAGE as described in the legend of Fig. 1. The relative rate of incorporation of [$^{35}$S]methionine into the 37-kD protein was measured by 2D densitometric scanning of fluorographs (RFT Scanning Densitometer) and compared with the labeling of an internal standard protein (43-kD) in the nerve medium (see asterisks in Fig. 2, a and d). The relative labeling of the 43-kD protein compared to the total amount of [$^{35}$S]methionine incorporated into the extracellular soluble proteins remained unchanged in the distal stump of sciatic nerves after axotomy.

in adult control (sham-operated) animals (see Fig. 2, a to c).

In fluorographs of labeled extracellular soluble proteins from intact control PNS and CNS tissue (Fig. 2, a to c), the synthesis of the 37-kD protein appeared to be low. However, within 10 to 14 days after a hemisection of the spinal cord (Fig. 2e) and after a crush-induced lesion of the optic nerve (Fig. 2f), the synthesis of the protein was increased to rates similar to those in crushed sciatic nerve (Fig. 2d). The 37-kD proteins in the PNS and CNS had an identical molecular weight and isoelectric point of *p*H 5.4, and their synthesis increased with a similar time course, which suggests that these proteins are homologous. When the protein-stained gels (Fig. 2, g to i) corresponding to the fluorographs in Fig. 2, d to f, were compared, the 37-kD protein was detected only in the sciatic nerve (Fig. 2g). In the distal stump of injured rat sciatic nerve, the protein accumulated to approximately 2 to 5 percent of the total extracellular protein (*7*). Despite its high rate of synthesis, the 37-kD protein did not accumulate in rat spinal cord (Fig. 2h) or optic nerve (Fig. 2i). Even prolonged synthesis of the protein at its maximum rate for at least 3 months after crushing the optic nerve did not increase its concentration of this protein.

The rapid disappearance of the 37-kD protein from the extracellular space in spinal cord and optic nerve may be due to proteolytic degradation, because uptake (or reuptake) and intracellular accumulation of the protein in these CNS tissues was not observed. A similar degradative mechanism was either absent or inhibited in the peripheral nerve.

The effect of permanent denervation and axonal regeneration on the synthesis of the 37-kD protein in rat sciatic nerve was also examined. In one group of adult Sprague-Dawley rats, sciatic nerves were unilaterally transected with a permanent separation of the proximal and distal nerve stumps. In another group, sciatic nerves were unilaterally crushed. The nerve stumps distal to the site of lesion were removed between 1 and 8 weeks after injury, and extracellular soluble proteins were labeled with [$^{35}$S]methionine and analyzed by 2D PAGE and fluorography as described for developing nerves. The relative rate of [$^{35}$S]methionine incorporation into the 37-kD protein was estimated by densitometric scanning of the fluorographs. The synthesis of the protein increased to similar maximum rates in both crushed and transected sciatic nerves within 1 week after injury. In nerves with crush lesions that allowed subsequent axon elongation from the proximal into the distal nerve stump, the rate of 37-kD protein synthesis declined slowly to rates near control within 8 weeks after the lesion. However, in a transected sciatic nerve where regeneration of axons into the distal stump was prevented, the synthesis of the protein did not return to the low control rates but remained at a maximum rate for at least 8 weeks after injury. Further, experiments on the exact spatial distribution of the sites of synthesis of the 37-kD protein in longer sciatic nerves from rabbits (*10*) revealed that it is synthesized exclusively in those segments of a severed nerve where the axons degenerate (*1*). These results confirm the earlier observation (*7*) but suggest further that regenerating axons in sciatic nerve may provide a signal to decrease the synthesis of the 37-kD protein in nerve. The determination of the cellular origin of the protein should be important in exploring the nature of this signal.

The expression of a 37-kD protein during development as well as after injury of the PNS and CNS suggests that it plays a role in de novo nerve growth and in nerve repair. The potential to increase the rate of synthesis and secretion of the 37-kD protein after axotomy was retained in both the mature PNS and CNS. However, the protein accumulated only in the PNS. The lack of accumulation of the protein in the adult mammalian CNS may represent one of the critical molecular differences between PNS and CNS that limit the ability of central axons in mammals to regenerate. Although the function of the 37-kD protein in nerve development or regeneration is still unknown, the time course and spatial distribution, as well as the possibility that its synthesis is axon-regulated, favor a function related to some aspects of nerve growth and maturation that require contact between axons and their sheath cells (*11*).

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## Genetic Origin of Mutations Predisposing to Retinoblastoma

Abstract. *Retinoblastoma is one of several human tumors to which predisposition can be inherited. Molecular genetic analysis of several nonheritable cases has led to the hypothesis that this tumor develops after the occurrence of specific mitotic events involving human chromosome 13. These events reveal initial predisposing recessive mutations. Evidence is presented that similar chromosomal events occur in tumors from heritable cases. The chromosome 13 found in the tumors was the one carrying the predisposing germline mutation and not the homolog containing the wild-type allele at the* Rb-1 *locus. These results suggest a new approach for identifying recessive mutant genes that lead to cancer and a conceptual basis for accurate prenatal predictions of cancer predisposition.*

Retinoblastoma, a childhood tumor of embryonic neural retina, occurs in heritable and nonheritable forms. Genetic and physical mapping data implicate the *Rb-1* locus on human chromosome 13 in the development of both forms of the tumor (*1–6*). Knudson has proposed that the conversion of a normal retinal cell to a malignant retinoblastoma cell requires two events (*7–9*). In this model, heritable