ture birds (*15*). The discovery of hair cell regeneration, with replacements originating from unidentified stem cells or cells that are ordinarily postmitotic, suggests that the possibility of self-repair after trauma should not be ruled out in mammalian ears and other neuroepithelial derivatives simply because their cells are mitotically quiescent in normal postembryonic life.

REFERENCES AND NOTES

1. T.-J. Yoo, in *Neurobiology of Hearing: The Cochlea*, R. A. Altschuler, D. W. Hoffman, R. P. Bobbin, Eds. (Raven, New York, 1986), pp. 425–440; L. P. Rybak, *ibid.*, pp. 441–454; D. W. Nielsen and N. Slepecky, *ibid.*, pp. 23–46; G. Bredberg, *Acta Oto-Laryngol. Suppl.* **236**, 1 (1968).
2. R. J. Ruben, *Acta Oto-Laryngol. Suppl.* **220**, 1 (1967); A. Katayama and J. T. Corwin, *Soc. Neurosci. Abstr.* **12**, 1048 (1986).
3. J. T. Corwin, *J. Comp. Neurol.* **201**, 541 (1981); *ibid.* **217**, 345 (1983); *Proc. Natl. Acad. Sci. U.S.A.* **82**, 3911 (1985).
4. D. A. Cotanche, J. C. Saunders, L. G. Tilney, *Assoc. Res. Otolaryngol. Abstr.* **9**, 14 (1986); D. A. Cotanche, *Hear. Res.* **30**, 181 (1987).
5. M. Makaretz, M. E. Schneider, J. C. Saunders, *Assoc. Res. Otolaryngol. Abstr.* **10**, 117 (1987).
6. R. M. Cruz, P. R. Lambert, E. W. Rubel, *Arch. Otolaryngol.* **113**, 1058 (1987).
7. Nineteen White Leghorn chickens in eight groups of two to four individuals were placed in a small wire cage with food and water and exposed to acoustic overstimulation for 48 hours as described in the text and in (*4*). At the start of the stimulation the chicks were 9 to 13 days old, ensuring that the cells in their cochleae would have been mitotically quiescent for 3 weeks (*9*). Immediately after the stimulation one chick from each group was overanesthetized and its cochleae were fixed in 1% osmium tetroxide and prepared for scanning electron microscopy (SEM) by standard methods. Other cochleae were prepared for SEM at 6, 8, and 10 days after overstimulation. The remaining chicks received [*methyl*-^{3}H]thymidine (ICN) by intramuscular injections of 1.5 μCi per gram of body weight given three times each day for 10 days (two chickens) or from two Alzet minipumps which were implanted subcutaneously while chicks were under anesthesia and which delivered isotope continuously at approximately 3 μCi per gram of body weight per day. Two controls received isotope but were not exposed to overstimulation. All of the isotope-treated chickens were maintained for 9 to 10 days after overstimulation (after the start of the isotope treatment for controls), then they were overanesthetized and their cochleae were fixed in buffered 3% glutaraldehyde. Transverse serial sections of paraffin- or epoxy-embedded cochleae were mounted on microscope slides that were later dipped in Kodak NTB2 liquid photographic emulsion and processed (*3*). A cell was scored as labeled if four or more silver grains were present above the nucleus.
8. D. A. Cotanche and K. K. Sulik, *Dev. Brain Res.* **16**, 181 (1984); L. G. Tilney, M. S. Tilney, J. C. Saunders, D. J. De Rosier, *Dev. Biol.* **116**, 100 (1986).
9. A. Katayama and J. T. Corwin, *Assoc. Res. Otolaryngol. Abstr.* **11**, 56 (1988).
10. J. T. Corwin, in *The Biology of Change in Otolaryngology*, R. J. Ruben, T. R. Van De Water, E. W. Rubel, Eds. (Elsevier, New York, 1986), pp. 291–304.
11. K. J. Balak and J. T. Corwin, *Assoc. Res. Otolaryngol. Abstr.* **11**, 107 (1988); J. T. Corwin, K. J. Balak, P. C. Borden, in *Neurobiology and Evolution of the Lateral Line System*, S. Coombs, P. Gorner, H. Munz, Eds. (Springer-Verlag, New York, in press).
12. K. J. Balak and J. T. Corwin, unpublished observation.
13. T. R. Van De Water and R. J. Ruben, *Acta Oto-Laryngol.* **95**, 470 (1983); J. T. Corwin, *J. Comp. Neurol.* **239**, 445 (1985).
14. J. C. Saunders and L. G. Tilney, in *New Perspectives on Noise-Induced Hearing Loss*, R. P. Hamernik, D. Henderson, R. Salvi, Eds. (Raven, New York, 1982), pp. 229–248; J. C. Saunders and N. Coppa, in *Contributions to Sensorineural Hearing Loss*, M. J. Collins, T. J. Glattke, L. A. Harker, Eds. (Univ. of Iowa Press, Iowa City, 1986), pp. 29–58.
15. B. M. Ryals and E. W. Rubel, *Science* **240**, 1774 (1988).

16. We thank P. Borden for technical assistance and K. Balak, M. E. Bitterman, I. M. Cooke, F. C. Greenwood, R. Hammer, S. Palumbi, and C. Womersley for comments. Supported by a Research Career Development Award and grant NS19061 from the National Institute of Neurological and Communicative Disorders and Stroke (J.T.C.), by a grant from the Deafness Research Foundation (D.A.C.) and by USPHS grant RCMI-RR03061 to the University of Hawaii. A brief report of these findings authored by J. T. Corwin and D. A. Cotanche is in *Soc. Neurosci. Abstr.* **13**, 539 (1987).

11 January 1988; accepted 15 April 1988

Hair Cell Regeneration After Acoustic Trauma in Adult *Coturnix* Quail

BRENDA M. RYALS AND EDWIN W. RUBEL

Recovery of hair cells was studied at various times after acoustic trauma in adult quail. An initial loss of hair cells recovered to within 5 percent of the original number of cells. Tritium-labeled thymidine was injected after this acoustic trauma to determine if mitosis played a role in recovery of hair cells. Within 10 days of acoustic trauma, incorporation of [^{3}H]thymidine was seen over the nuclei of hair cells and supporting cells in the region of initial hair cell loss. Thus, hair cell regeneration can occur after embryonic terminal mitosis.

PROLIFERATION, MIGRATION, AND differentiation of sensory hair cells occur embryonically or during early-postnatal development in mammals and birds. Once cell division ends, the capacity to produce new cells is thought to be lost for the duration of life, and hair cells that are lost as a result of toxic or traumatic events cannot be replaced. Certain bony fishes and amphibians, however, can produce hair cells throughout life and presumably replace any that are lost as a result of trauma or aging (*1*). In young birds, hair cell loss is reduced over time after ototoxicity and acoustic trauma (*2*), and stereocilia are replaced after acoustic trauma (*3*). This evidence suggests that the potential for hair cell regeneration after trauma may exist in young birds. In older birds (*4*) recovery of hair cell stereocilia does not occur, suggesting that the recovery process is age related and signifying that there may be a critical period during which recovery from acoustic trauma is possible. Another consideration, however, is that more extensive destruction of hair cells in the older birds renders the system incapable of significant repair (*5*).

In our study the following questions were addressed: (i) Is recovery from ototoxic agents or acoustic trauma related to the age at which acoustic overstimulation occurs or is it related to the extent of the trauma? (ii) How is the recovery taking place? Are new cells regenerated to replace lost hair cells? In the first experiment, we determined if recovery of lost hair cells can occur in adult birds. Sexually mature, adult quail (*Coturnix coturnix*) were continuously exposed for 12 hours to a 115-dB pure tone (*6*). Adult quail were used to determine if recovery stemmed from some process unique to early development, and 115-dB stimulation was used to optimize the probability of recovery. Survival time after acoustic trauma was varied from 0 to 60 days after exposure (*7*). At the appropriate time the birds were killed and their cochleae fixed, dissected free, osmicated, embedded in plastic, sectioned in the transverse plane, and stained. The number of hair cells extending across the basilar membrane was counted at each 100-μm interval from the basal to the apical end (*8*).

Ten days after the acoustic trauma the number of hair cells had decreased markedly (*9*) through the basal and middle portion of the cochlea (Fig. 1). In the middle of the cochlea as many as 70% of the hair cells were lost. In birds exposed to the same acoustic trauma but allowed to survive 30 days, hair cell loss was much less dramatic. Thirty days after acoustic trauma, the position of the maximum hair cell loss, at about the middle of the cochlea, was similar to that of the 10-day survival group, but the extent had decreased from 70% to only about 31%. For birds allowed to survive a longer time (60 days), there was evidence of a continued progression toward a return to the normal complement of hair cells. No

B. M. Ryals, Audiology and Speech Pathology, Veterans Administration Medical Center, Richmond, VA 23249. E. W. Rubel, Department of Otolaryngology, University of Washington, Seattle, WA 98195.

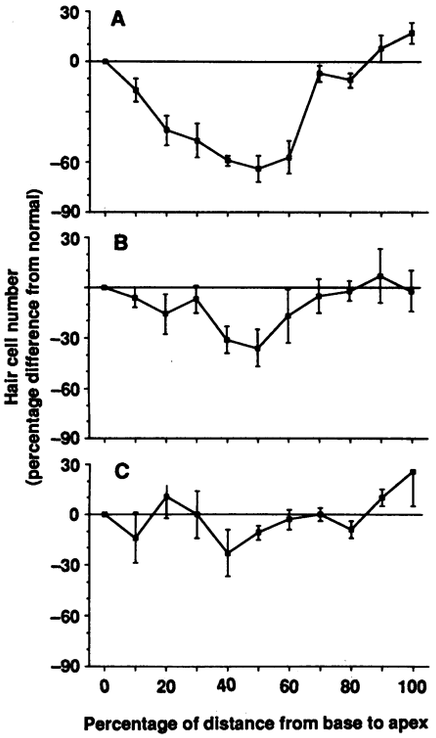

Fig. 1. The mean percentage difference in hair cell number, as compared to normal controls (n = 6), after acoustic trauma for (**A**) 10-day, (**B**) 30-day, and (**C**) 60-day survival times. Normal hair cell number in controls is shown by a straight line at 0 along the cochlea from base to apex. Average percentage difference in hair cells from normal (±1 SEM) is shown in 10% intervals along the cochlea from base to apex at each survival time (n = 6, 5, and 3 for 10-day, 30-day, and 60-day survival, respectively).

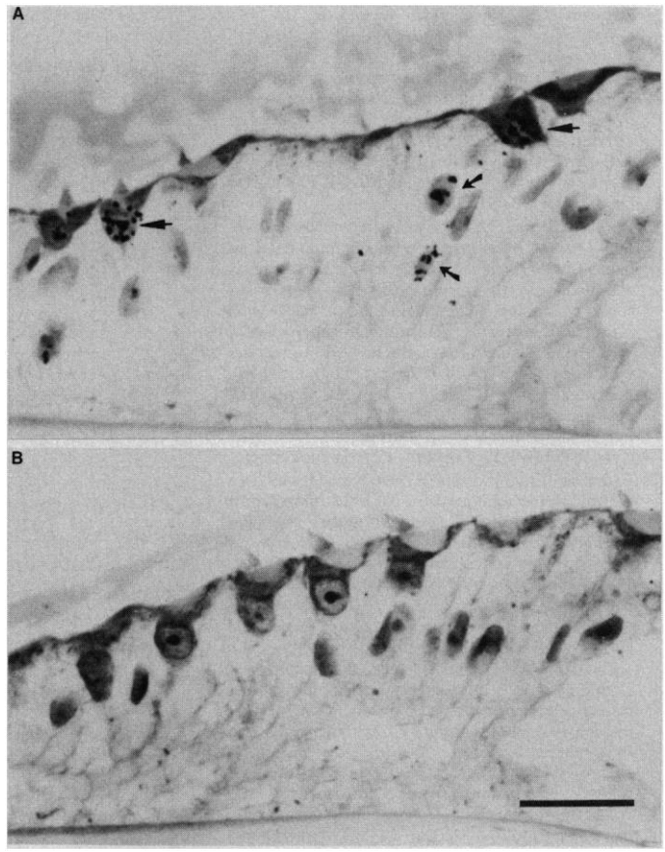

Fig. 2. (**A**) Photomicrograph of hair cells located within the damaged area of the basilar membrane of a sound-exposed animal. [^{3}H]thymidine labeling is clearly seen above the nucleus of short hair cells (large arrowheads) and above supporting cells (small arrows). (**B**) Photomicrograph of a similar region of the basilar membrane from a control animal that was not sound exposed but was given the same [^{3}H]thymidine injections. No labeled hair cells or supporting cells are seen. Uptake of thymidine was similar in the two animals as indicated by the number of labeled red blood cells (not shown). Bar, 20 μm.

reduction in cell number was seen in the undamaged area when the cell number in the damaged area increased. Further, measures of cell size showed no obvious change in the size of hair cells counted (*9*).

Finally, if cellular migration had occurred and if it accounted for the replacement of hair cells in the damaged area, the total number of cells throughout the papilla should not have changed. A comparison of all three survival groups showed significant recovery of total hair cell number between 10 and 60 days after acoustic trauma (*9*). Thus recovery of hair cell number does occur in mature, adult birds after acoustic trauma and does not appear to be restricted to some critical time during early development.

In the second experiment, we tested whether recovery of the number of hair cells represents actual regeneration of new cells from a dormant or dedifferentiated stem cell population. Sexually mature adult quail were exposed to the same acoustic trauma as in the first experiment. Immediately after acoustic trauma the birds received a series of injections of [^{3}H]thymidine (*10*). Control birds, not exposed to acoustic trauma, were given the same injections and housed in the same room. On the 10th day after acoustic trauma the birds were killed and their basilar papillae removed and prepared for serial sectioning and autoradiography (*11*).

The autoradiographic procedures produced low levels of background. In the control birds, collections of 10 to 20 grains could be seen over red blood cells, but no labeled hair cells or support cells were ever observed (more than 200 sections were examined throughout the cochleae) in any of the five control birds. In the sound-exposed birds, hair cell loss and damage were observed in the basal to middle section of the papilla comparable in position and amount to that described for the first experiment. Incorporation of [^{3}H]thymidine was clearly seen over hair cells and support cells beginning at about 30% of length and continuing throughout the damaged area (Fig. 2A). This pattern of labeling was seen in all five of the sound-exposed birds. No [^{3}H]thymidine labeling was observed outside the damaged area. The region of most cell loss was located on the inferior edge of the basilar membrane from 30 to 50% of length. Within the damaged area the most consistent [^{3}H]thymidine labeling was seen over cells midway between the superior and inferior edges of the basilar membrane. This section is generally considered a transition zone from tall to short hair cells (*12*). Both tall and short hair cells as well as support cells were labeled; generally, short hair cells were labeled more often than tall hair cells. Extensive damage and hair cell loss were still observed adjacent to labeled cells.

Corwin and Cotanche (*13*) in a study with thymidine labeling in young chicks, also observed labeled hair cells after noise exposure, suggesting mitotic activity in postmitotic cells. Our study and their study demonstrate hair cell regeneration after terminal mitosis in the inner ear of birds. In addition, our study shows that the regenerative potential is retained in adult animals, suggesting that a dormant stem cell population is retained throughout life. Although the location and mechanism of activation of these precursor cells are yet to be identified, the potential may exist to restore inner ear sensory elements after injury or disease.

REFERENCES AND NOTES

1. J. T. Corwin, *J. Comp. Neurol.* **201**, 541 (1981).
2. R. M. Cruz, P. R. Lambert, E. W. Rubel, *Arch. Otolaryngol.* **113**, 1058 (1987); E. W. Westbrook, B. M. Ryals, W. Lippe, *Assoc. Res. Otolaryngol. Abstr.* **11**, 50 (1988); M. Makaretz, M. E. Schneider, J. C. Saunders, *ibid.* **10**, 149 (1987).
3. D. A. Cotanche, J. C. Saunders, L. G. Tilney, *Assoc. Res. Otolaryngol. Abstr.* **9**, 16 (1986); D. A. Cotanche, *Hear. Res.* **30**, 181 (1987).
4. W. J. Henry, M. Makaretz, M. Schneider, J. C.

Saunders, *Otolaryngol. Head Neck Surg.* **97**, 136 (1987).
5. In our previous studies of hair cell loss after acoustic trauma in young chicks, we reported a gradual increase in the amount of hair cell loss over a period from 10 to 30 days after trauma with no further increase in cell loss after 30 days. In these animals a 125-dB pure tone [5 dB stronger than that used in (*4*)] was used [E. W. Rubel and B. M. Ryals, *Acta Oto-laryngol.* **93**, 31 (1982)].
6. B. M. Ryals and E. W. Rubel, *Acta Oto-laryngol.* **93**, 205 (1982). Birds were partially immobilized, in pairs, in wire mesh cages designed for this purpose, and placed directly in front of a 200-W speaker in a sound booth. Decibel levels near the ear canals were measured just before stimulation and at cessation of stimulation. Calibration of the acoustic signal was performed with a 2.5-cm electret microphone with a preamplifier to a B&K 2033 signal analyzer. The acoustic environment of the wire mesh cages varied by no more than ±3 dB throughout; harmonics were at least 40 dB below the experimental tone.
7. Fourteen 3- to 6-month-old quail were exposed to a 1500-Hz pure tone at 115 dB for 12 hours. After noise exposure they were returned to their cages and allowed to survive for 10, 30, or 60 days. One ear from each animal was analyzed for hair cell number: 10-day survival, $n = 6$; 30-day survival, $n = 5$; 60-day survival, $n = 3$.
8. Groups of three or four sections (3 μm thick) were collected at each 100-μm interval throughout the cochlea, mounted in serial order, and stained with Toluidine Blue. Quantitative analysis of the number of hair cells was performed by viewing each section under a 40× planapochromatic oil immersion objective (numerical aperture, 1.0) at a total magnification of ×500. The criteria for the presence of a hair cell were presence of cuticular plate, stereocilia, and cell body.
9. No statistical difference was found in hair cell size (short hair cells, 11.9 μm; tall hair cells, 6.06 μm) between the noise-exposed and control birds [short hair cells, $F(3,52) = 2.30$, $P > 0.05$; and tall hair cells, $F(3,52) = 1.98$, $P > 0.05$]. An additional analysis of cell density by means of scanning electron microscopy ($n = 2$) showed no obvious change in cell density outside the damaged area. Finally, statistical comparisons were made on total number of hair cells. The average total number of hair cells (±1 SEM) in each group was as follows: control, 275 ± 6.2; 10 day, 188 ± 8.5; 30 day, 236 ± 18.9; and 60 day, 255 ± 8.1. An F test for simple randomized design showed a significant difference between control and experimental groups: $F(3,16) = 11.73$, $P < 0.001$. Individual comparisons with the t test for differences among several means [J. L. Bruning and B. L. Kintz, *Computational Handbook of Statistics* (Scott, Foresman, Glenview, IL, 1968)] revealed least significant differences at the 0.01 level and at the 0.05 level: between normal controls and the 10-day survival group ($P < 0.01$); between 10- and 60-day survival group ($P < 0.01$); between normal controls and the 30-day survival group ($P < 0.05$); and between the 10- and 30-day survival group ($P < 0.05$). Thus recovery of cells could not be accounted for by migration of undamaged cells into the damaged area.
10. Ten 4-month-old adult quail were used. Five birds were exposed to the 1500-Hz, 115-dB acoustic trauma, and five birds were used as age-matched controls. Control and experimental birds received two intramuscular injections of [^{3}H]thymidine daily for 10 days after noise exposure; controls were paired with experimental animals for injections. Each injection consisted of 380 μCi of [^{3}H]thymidine [84 Ci per millimole of [^{3}H]thymidine in 0.38 ml of sterile distilled water (0.001 Ci/ml) (NEN). This amount corresponds to approximately 2.5 μCi per gram of body weight, a dose similar to that used by other investigators studying adult birds [G. D. Burd and F. Nottebohm, *J. Comp. Neurol.* **240**, 143 (1985)].
11. Cochleae were embedded and sectioned serially as in (*8*). Sections (3 μm thick) were mounted on acid-cleaned slides in basal to apical order and prepared for autoradiography by using Kodak MTB-2 emulsion and routine methods. After exposure for 4 to 7 weeks, the autoradiographs were developed in D19 and stained through the emulsion with Toluidine Blue.
12. T. Takasaka and C. A. Smith, *J. Ultrastruct. Res.* **35**, 20 (1971); K. Tanaka and C. A. Smith, *Ann. Otol. Rhinol. Laryngol.* **84**, 287 (1975).
13. J. T. Corwin and D. A. Cotanche, *Soc. Neurosci. Abstr.* **13**, 539 (1987); J. T. Corwin and D. A. Cotanche, *Science* **240**, 1772 (1988).

14. We thank E. Westbrook for assistance in dissection and specimen preparation, B. Adelman for advice and assistance in handling radioactive isotopes, and J. Debell for developing specimens for autoradiography. Supported by Veterans Administration Rehabilitation Research and Development and NIH grant NS24522.

21 January 1988; accepted 27 April 1988

A Point Mutation in the c-*myc* Locus of a Burkitt Lymphoma Abolishes Binding of a Nuclear Protein

MARIA ZAJAC-KAYE,* EDWARD P. GELMANN, DAVID LEVENS

A 20–base pair region in the first intron of the human c-*myc* gene was identified as the binding site of a nuclear protein. This binding site is mutated in five out of seven Burkitt lymphomas sequenced to date. To investigate the protein-recognition region in greater detail, the abnormal c-*myc* allele from a Burkitt lymphoma line (PA682) that carries a t(8;22) chromosomal translocation was used. A point mutation in the binding region of the PA682 c-*myc* DNA abolished binding of this nuclear protein. This protein may be an important factor for control of c-*myc* expression, and mutations in its recognition sequence may be associated with c-*myc* activation in many cases of Burkitt lymphoma.

IN BURKITT LYMPHOMA (BL), THE c-*myc* allele present on the normal chromosome 8 is transcriptionally silent, while the c-*myc* locus located on the translocated chromosome is activated (*1*). The c-*myc* transcriptional activation has been suggested to result from juxtaposition of the c-*myc* with an immunoglobulin gene regulatory sequence (*2*), from truncation within the gene (*3*), or from mutation in the exon I and/or its 5′ flanking sequence (*4, 5*). However, the relative contributions of the above mechanisms to c-*myc* expression in BL remain controversial. Cell line PA682 is an Epstein-Barr virus (EBV)–positive BL cell line derived from a patient with acquired immunodeficiency syndrome (AIDS) (*6*), which contains a t(8;22) chromosomal translocation. In this cell line the c-*myc* gene has not been rearranged and the translocation occurred at least 16 kb downstream of the c-*myc* gene (*7*). The c-*myc* gene in PA682 is activated, as demonstrated by ribonuclease (RNase) protection experiments in which transcription of the c-*myc* gene was shown to occur predominantly from the abnormal allele (*8*). We isolated from PA682 cells two different c-*myc* gene loci corresponding to the normal and abnormal alleles. The two alleles could be distinguished by an approximately 40-nucleotide deletion at the 5′ end of the first intron as determined by restriction enzyme mapping (*7*). Clone λ-PA22, representing the abnormal allele, was subcloned, and the nucleotide sequence for the first exon, part of the first intron, as well as 80 bp of the 5′ flanking region, was determined (Fig. 1). Comparison of the sequences from the abnormal c-*myc* allele of the λ-PA22 clone with the normal c-*myc* gene (*9*) revealed mutations (base substitutions as well as single base deletions) scattered throughout the 3′ region of the first exon and the 5′ region of the first intron (Fig. 1). In addition, intron I contained a 38-bp deletion of a thymidine-rich sequence 9 bp downstream of the exon I splice-donor site.

Since the elongation of the c-*myc* mRNA, initiated at the P1 and P2 promoters located at the 5′ end of exon I, has been shown to be blocked in the vicinity of the exon I–intron I boundary (*10, 11*), we examined the read-through efficiency of c-*myc* transcripts in PA682 cells. If the elongation of the c-*myc* mRNA transcription is attenuated or terminated because of the thymidine runs (T_4 T_3 T_7) present in the first intron, then deletion of the poly T segments should abrogate the block to c-*myc* RNA elongation. We performed nuclear run-off experiments on PA682 cells and used human cord blood (CB) cells immortalized with the strain of EBV derived from PA682 cells as a control. We examined transcription of the first and second exons using single-stranded probes for each DNA strand (Fig. 2B). Both the

M. Zajac-Kaye and E. P. Gelmann, Medicine Branch, Clinical Oncology Program, National Cancer Institute, Bethesda, MD 20892.
D. Levens, Laboratory of Pathology, National Cancer Institute, Bethesda, MD 20892.

*To whom correspondence should be addressed.