axotomy, or cell culture. Our previous studies have shown that, once formed, certain of these electrical connections are selectively stabilized, while others are eliminated (*2, 3*). Thus while specialized mechanisms can refine neuronal circuits, growth-related processes can act as simple screening mechanisms (*3*) to set the stage for such refinement.

ROBERT D. HADLEY*
S. B. KATER
CHRISTOPHER S. COHAN
Department of Zoology,
University of Iowa, Iowa City 52242

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11. In cell culture, neurons from buccal, pedal, and cerebral ganglia have been found to couple to one another as well as to neuron 5.
12. "Stable" neurons were those that showed no apparent continuation of outgrowth for at least 2 days. In some cases, neurons judged to be stable continued to make some small changes at their endings.
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* Present address: Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, Mo. 63110.

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Minor Histone 2A Variants and Ubiquinated Forms in the Native H2A:H2B Dimer

Abstract. *Histone octamers from calf thymus were separated into $(H3:H4)_2$ tetramers and H2A:H2B dimers by chromatography through Sephadex G100. The tetramers and dimers were analyzed for variants, ubiquitin adducts, and proteolyzed forms. The minor histone variants H2A.X and H2A.Z were found to be associated with histone H2B as H2A.X:H2B and H2A.Z:H2B dimers, respectively. Ubiquitin adducts of the H2A's and H2B were also present in H2A:H2B dimers.*

Histones mediate the primary condensation of DNA in the eukaryotic chromosome. Brief digestion of chromatin with micrococcal nuclease yields particles called nucleosomes, each of which contains about 200 base pairs of DNA, two molecules of each of the core histones (H2A, H2B, H3, and H4), plus one molecule of H1. Further digestion of chromatin leads to the production of the core nucleosome particle, which consists of 146 base pairs of DNA wrapped around the histone octamer (*1*). The octamer, which is extracted from chromatin with 2*M* NaCl, is formed by the association of two H2A:H2B dimers with an $(H3:H4)_2$ tetramer (*2*). The primary sequences of histones within each class of core histone may have a subtle effect on the structure of the core nucleosome. In vertebrates, histones H3, H2B, and H2A can have nonallelic variants, which are related by simple amino acid substitutions (*3*). Newrock *et al.* (*4*) showed that the patterns of nonallelic variants may change during embryogenesis in sea urchins. Simpson (*5*) showed that such changes in the expression of histone variants during development are accompanied by changes in the stability of the nucleosome core particle during thermal denaturation and deoxyribonuclease digestion.

For mammalian H2A's, the situation is more complex. West and Bonner (*6*) identified eight protein species within the histone 2A family of proteins. The major variants are H2A.1 and H2A.2, and the minor variants are H2A.X and H2A.Z. The other four species are ubiquitin conjugates of each of these variants. These proteins are clearly separable on a two-dimensional electrophoretic system having an acid-urea-Triton (AUT) acrylamide gel in the first dimension and an acid-urea-cetyltrimethylammonium bromide (AUC) acrylamide gel in the second dimension (*7*). The H2A variants X and Z do not comigrate with H2A.1 or H2A.2 in sodium dodecyl sulfate (SDS)-polyacrylamide gels, but both contain a conserved peptide that has been found in all H2A's sequenced to date. H2A.Z is of particular interest because it is quite different from other H2A's as shown by peptide mapping, but it is more conserved during evolution (*8*). The synthesis of the major H2A variants, H2A.1 and H2A.2, as well as the H3 variants, H3.1 and H3.2, is restricted to the S phase of the cell cycle. In contrast, the minor variants of these two core histones—H2A.X, H2A.Z, and H3.3—are synthesized in small amounts throughout the S, G_2, and G_1 phases of the cell cycle (*9*). West and Bonner (*6*) showed that H2A.X and H2A.Z are associated with the nucleosome core particles released by digestion of nuclei with micrococcal nuclease. However, whether H2A.X and H2A.Z function like the major H2A variants in the nucleosome has not been directly tested. We now report that these minor variants of H2A are associated with the core histone octamer and that they subsequently fractionate with the H2A:H2B dimer. In addition, the ubiquitinated (ubiquitin-conjugated) forms of the H2A variants and H2B comigrate with the H2A:H2B dimer.

The proteins released by extraction of calf thymus chromatin with 2*M* NaCl, *p*H 7.5 (after an earlier extraction with 0.35*M* NaCl), were chromatographed through a Sephadex G100 column in the presence of 2*M* NaCl, 10 m*M* tris, and 1 m*M* EDTA, *p*H 7.5. The peak fractions were pooled and concentrated at reduced pressure to 15 mg/ml.

The histone octamer exists in equilibrium with its subunits, the $(H3:H4)_2$ tetramer and the H2A:H2B dimer, and the balance of the equilibrium is affected by the ionic strength, *p*H, temperature, and presence of urea (*2*). We were therefore able to use gel chromatography of "octamer" through Sephadex G100 in the same ionic strength (2*M* NaCl) conditions, but at a reduced *p*H (5.0), to fractionate the $(H3:H4)_2$ tetramer and the H2A:H2B dimer. The histones in each fraction were subjected to electrophoresis in a two-dimensional AUT-AUC gel system (*7*). Figure 1 shows the elution profile from the Sephadex G100

column and the protein composition of the indicated fractions from this profile. Figure 1C shows the peak fraction of native tetramer and Fig. 1E shows the peak fraction of native dimer. Like the major H2A variants, H2A.1 and H2A.2, the minor H2A variants, H2A.X and H2A.Z, are also maximum at the peak of the H2A:H2B dimer. Therefore we conclude that H2A.X and H2A.Z are found in octamers and associate with H2B as do H2A.1 and H2A.2.

H2A.X is ubiquitinated, phosphorylated, and acetylated like the major H2A variants, and they have many tryptic peptides in common (*6, 10*). Its association with H2B in the native H2A:H2B dimer substantiates this similarity. Although H2A.Z contains a conserved peptide (residues 21 to 29) that is found in all sequenced H2A's and has a ubiquitin adduct, it is not phosphorylated as the other H2A's are and it is acetylated differently (*6, 10*). In addition, it is much more conserved during evolution than the other H2A's (*8*). These differences suggest that H2A.Z may be functionally differentiated from the other H2A's. The occurrence of H2A.Z in the native H2A:H2B dimer indicates that H2A.Z substitutes for the other H2A's in the nucleosome and may confer a differentiated function on a nucleosome that contains it.

About 10 percent of the major H2A variants in mammals are covalently linked to ubiquitin, an 8000-dalton protein (*11*), forming the conjugate A24. The minor H2A variants, H2A.X and H2A.Z, are ubiquitinated to a similar extent (*6*), and H2B is ubiquitinated to a lesser extent (*12*). Protein A24 has been found in nucleosome cores and may be cross-linked to H2B (*13*). The positions of these ubiquitinated histones are shown in Fig. 1E; because of its small amount and juxtaposition to the major variants, ubiquitinated H2A.X is not visible in this figure. The ubiquitin adduct of H2B (u2B) is clearly present. The ubiquitinated histones fractionate with the H2A:H2B dimer, but because of their increased molecular weight as compared with their nonubiquitinated forms, their elution is maximum at a position slightly to the "heavy" side of the H2A:H2B peak (Fig. 1, D to F). This direct demonstration of the ubiquitinated histones in H2A:H2B dimers substantiates earlier results for A24 and extends these results to include the ubiquitin conjugates of H2A.Z and H2B.

Since H2A.X and H2A.Z are usually present in small amounts, and especially in calf thymus, it was necessary to load the polyacrylamide gels with a large amount of the major variants in order to see the minor ones. For this reason, small amounts of other products are visible, all of which can be explained as enzymatically or chemically altered histones. Histone 2A is cleaved by the H2A-specific protease, which in 2*M* NaCl recognizes and cleaves a specific site on the molecule between the valine at residue 114 and the leucine at residue 115 (*14*). The spots in Fig. 1E labeled c2A, with lines leading to .1 and .2, demonstrate low activity of this protease on H2A.1 and H2A.2, respectively, during the purification. The spots labeled c2B include several proteolyzed forms of H2B. These proteolyzed forms of H2A and H2B are maximum in Fig. 1F, indicating that they were present before column chromatography and subsequently fractionated from native dimers on the column.

The pattern of H3 spots in these gels is slightly more complicated because of the susceptibility of this core histone to partial proteolysis (*15*) and the presence in calf thymus H3 of cysteine residues (*16*). Just as the partially proteolyzed forms of H2A and H2B are eluted slightly later than their native counterparts, the partially proteolyzed forms of H3.1 (the group of smaller spots directly above c3.1) increase in relative amount when compared to native H3.1 (3.1) as the tetramer peak disappears. The fact that the highest proportion of H3 dimers formed before the first-dimension electrophoresis appeared in the early eluting tetramer fractions (Fig. 1B, spots p and q) suggests that, in a small fraction of the material eluting from the column at this

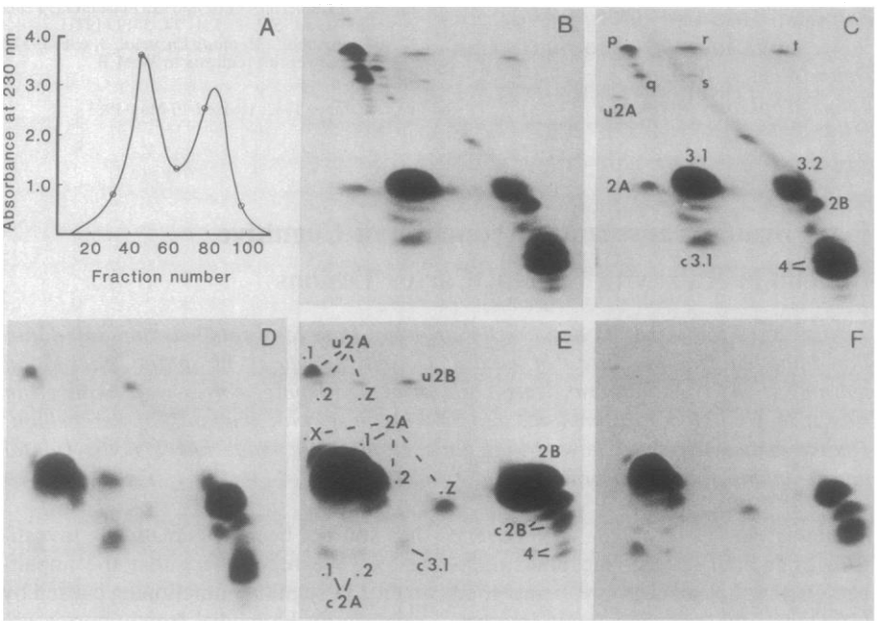

Fig. 1. (A) The $(H3:H4)_2$ tetramer and the H2A:H2B dimer in a pool of core histone octamer were fractionated by chromatography through a Sephadex G100 column eluted with 2*M* NaCl, 10 m*M* sodium phosphate, and 1 m*M* EDTA, *p*H 5.0. All purification steps were conducted at 4°C. Protein concentrations were determined by the biuret protein assay (*17*). Protein samples were collected from the indicated fractions of the column elution profile, desalted through small columns of Sephadex G25, eluted with 50 m*M* acetic acid, and then lyophilized to dryness. Histones were resolved in a two-dimensional electrophoretic system (*7*). The first dimension is an acid-urea-Triton X-100 (AUT) polyacrylamide gel, and the second dimension is an acid-urea-cetyltrimethylammonium bromide (AUC) polyacrylamide gel. The only gel modifications were the use of 8*M* urea and 10 percent acrylamide in the first dimension. Methylene blue tracking dye was run 5 cm into the resolving gel of the AUT first dimension while the free Coomassie blue stain was run 15 cm into the AUC resolving gel. (B to F) Second-dimension gels of the proteins shown in column fractions 33, 46, 65, 79, and 98, respectively. In (C), the abbreviations are *2A*, H2A.1 and H2A.2; *u2A*, ubiquitinated H2A.1 and H2A.2; *3.1*, H3.1; *c3.1*, slightly proteolyzed forms of H3.1; *3.2*, H3.2; *2B*, H2B; *4*, H4; *p*, H3.1 dimers formed before the first-dimension electrophoresis; *q*, dimers of c3.1 formed before the first-dimension electrophoresis; *r*, H3.1 dimers formed after the first-dimension electrophoresis; *s*, dimers of c3.1 formed after the first-dimension electrophoresis; and *t*, H3.2 dimers formed after the first-dimension electrophoresis. In (E), *u2A* with dashed lines to .1, .2, and .Z are the ubiquitinated forms of H2A.1, H2A.2, and H2A.Z, respectively; *2A* with dashed lines to .X, .1, .2, and .Z are the native forms of H2A.X, H2A.1, H2A.2, and H2A.Z, respectively; *c2A* with lines to .1 and .2 indicate the specific protease cleavage of H2A.1 and H2A.2, respectively; the other abbreviations are *u2B*, ubiquitinated H2B; *2B*, H2B; *c2B*, proteolyzed forms of H2B; *c3.1*, a proteolyzed form of H3.1; and *4*, H4.

point, two tetramers were joined together by a disulfide linkage and therefore fractionated from the bulk of the tetramer on the Sephadex column.

Thus, the minor H2A variants substitute for a fraction of the major H2A variants in the H2A:H2B dimer. This point is particularly relevant for H2A.Z because its sequence diverges considerably from those of the major H2A variants and because it differs from them in its pattern of modification. In addition, the finding that ubiquitination seems to have no effect on histone association is substantiated and is extended to include the minor H2A's and H2B.

CHRISTOPHER L. HATCH
Department of Biology, Johns Hopkins University, Baltimore, Maryland 21218
WILLIAM M. BONNER*
Laboratory of Molecular Pharmacology, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland 20205
EVANGELOS N. MOUDRIANAKIS
Department of Biology, Johns Hopkins University

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* Address reprint requests to W.M.B.

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Fetal Brain Transplants: Reduction of Cognitive Deficits in Rats with Frontal Cortex Lesions

Abstract. *Frontal cortex and cerebellar tissue from fetal rats was implanted into the damaged frontal cortex of adults. Cognitive deficits in spatial alternation learning that follow bilateral destruction of medial frontal cortex were reduced in rats with frontal cortex implants but not in those with implants of cerebellum. Histological evaluation showed that connections were made between the frontal cortex implants and host brain tissue.*

Interest in the problem of recovery from brain injury is growing, and a number of new approaches are being tried (*1*). One of the more novel and interesting of these involves the transplantation of embryonic brain tissue directly into the damaged brain of a mature recipient (*2*). In recent experiments the behavioral deficits associated with damage to the nigrostriatal or fimbria-fornix systems have been diminished by implanting fetal dopaminergic neurons or solid embryonic septal grafts, respectively, into the lesion sites (*3, 4*). Anatomical studies with anterograde and retrograde tracers have also shown that the transplants can establish connections with the host brain (*5*), while electrophysiological experiments show that the neural implants are capable of forming functional synapses (*6*).

Despite these achievements, the ability of brain grafts to mediate behavioral recovery after bilateral cortical ablations has still not been systematically investigated. We report here that the impairments in cognitive functioning caused by damage to the medial frontal cortex are significantly reduced by the implantation of fetal frontal cortex into the lesion site. Furthermore, injections of the enzyme horseradish peroxidase (HRP) show that the transplants and the host brains establish afferent neuronal connections.

Twenty-nine male Sprague-Dawley rats (Charles River; CD) approximately 105 days old at the time of surgery were used. Eight animals served as unoperated controls with sham incisions. The medial frontal cortex of the remaining 21 animals was damaged bilaterally by aspiration (*7*). Seven days after surgery 14 animals were implanted with fetal frontal cortex ($N = 8$) or fetal cerebellar tissue ($N = 6$) (*8*). (The unoperated controls and the seven lesion animals not receiving implants were anesthetized at this point and their wounds were reopened.) The transplanted neural tissue was obtained from CD rat fetuses on day 21 or 22 of gestation and placed into the cavity created by the removal of the medial frontal cortex (*9*). The implants had a volume of approximately 6 mm^3 and were placed bilaterally directly into the area of damage.

On the fourth day after transplantation all 29 animals began training on a spatial alternation task in a T-maze (*10*). Spatial alternation requires the water-deprived rat to enter the goal arm opposite the one entered on the previous trial in order to receive a 0.15-ml water reward. This test has been used to determine the effects of frontal cortex damage (*11*). Ten trials per day constituted a testing session, and animals were tested 5 days per week. When an animal made 19 of 20 choices correctly during two consecutive test sessions, or when 30 test sessions had been completed, testing was terminated for that rat. After behavioral testing, and between 78 and 155 days after transplantation, the rats that had received frontal cortex or cerebellar tissue were given injections of the retrograde transport marker HRP in the transplant or the host brain to determine whether afferent connections had been established between these neural regions (*12*).

We found that transplants of frontal cortex, but not cerebellar tissue, facilitated recovery from the lesions (Fig. 1). An analysis of variance revealed significant differences among the four groups in the number of days needed to meet our most stringent criterion, the making of 19 of 20 choices correctly in two consecutive days [$F(3, 25) = 10.91$, $P < 0.01$]. Randomization tests for two independent groups revealed that rats receiving frontal cortex performed significantly better than the lesion group that did not receive brain transplants in terms of number of days needed to make nine of ten choices correctly in 1 day ($P < 0.01$), number of days needed to make 18 of 20 choices correctly in two consecutive days ($P < 0.05$), total number of errors divided by number of trials needed to meet the most stringent criterion ($P < 0.05$), and number of perseverative errors divided by number of trials needed to meet the most stringent criterion ($P < 0.05$).

Animals that received frontal cortex scored significantly better than the group given cerebellar tissue on days needed to make nine of ten choices correctly in 1 day ($P < 0.05$) and number of perseverative errors divided by number of trials needed to make 18 of 20 and 19 of 20 choices correctly over 2-day periods