Chromatin Structure in Scrapie and Alzheimer's Disease

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ABSTRACT: Scrapie affected brains exhibit a number of pathological features in common with the human neurodegenerative condition, Alzheimer's disease. The present report describes studies on chromatin structure seen in these two disease processes.

Chromatin associated proteins influence transcriptional activity of DNA through an effect upon chromatin structure. We examined chromatin structure by: (1) measuring the capacity of the enzyme micrococcal nuclease to release mono- and dinucleosomes from isolated nuclei and (2) measuring DNA-histone interactions by examining the effect of ambient tonicity upon the release of chromatin proteins.

In two strains of mice infected with two strains of scrapie agent there was reduced accessibility to micrococcal nuclease and an increased content on dinucleosomes of the histone H1 and H1^{0} types. These changes precede clinical signs of scrapie and resemble those found in the human conditions of Alzheimer's and Pick's disease. Scrapie mouse brain differs from Alzheimer brain in that scrapie does not alter histone-DNA interactions as monitored by ionically induced histone release from chromatin. Despite similarities, the scrapie agent appears to operate upon different molecular mechanisms than those found in Alzheimer's disease.

RÉSUMÉ: La structure de la chromatine dans le scrapie et la maladie d'Alzheimer. Certaines manifestations pathologiques sont communes aux cerveaux atteints de scrapie et à ceux atteints de la maladie neurodégénérative humaine nommée maladie d'Alzheimer. Cet article décrit des études de la structure de la chromatine dans ces deux processus pathologiques.

Les protéines associées à la chromatine influencent l'activité de transcription de l'ADN par leur effet sur la structure de la chromatine. Nous avons examiné la structure de la chromatine par deux moyens: 1) en mesurant la capacité de la nucléase de micrococque de libérer des mono- est des dinucléosomes à partir de noyaux isolés; et 2) en mesurant les interactions de l'histone de l'ADN par l'examen de l'effet de la pression osmotique ambiante sur la libération des protéines de la chromatine.

Chez deux races de souris infectées par deux souches d'agent du scrapie, l'accessibilité à la nucléase de micrococque était réduite et le contenu en histone de type H1 and H1^{0} sur les dinonucléosomes était augmenté. Ces changements précèdent les signes cliniques du scrapie et ressemblent à ceux qu'on retrouve dans les affections de l'homme telles la maladie d'Alzheimer et la maladie de Pick. La différence entre le cerveau de souris atteint de scrapie et le cerveau atteint d'Alzheimer est que le scrapie ne modifie pas les interactions histone-ADN telle qu'en témoigne la libération de l'histone à partir de la chromatine, libération induite ioniquement. Malgré des similarités, l'agent du scrapie semble agir sur des mécanismes moléculaires différents de ceux qu'on retrouve dans la maladie d'Alzheimer.

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The unconventional transmissible encephalopathies of scrapie and Jakob Creutzfeldt share a number of features in common with Alzheimer's disease, a neurodegenerative condition of unknown etiology. In addition to the formation, in certain species, of neuritic plaques with amyloid cores[1,2] both conditions are progressive encephalopathies associated with neuron loss without inflammatory pathology.[3] The recent discovery of a mRNA transcript differentially expressed in both scrapie and in Alzheimer's disease compared to control brain supports the hypothesis that whatever the primary pathogenic event which initiates Alzheimer's disease, both etiological agents may induce expression of a common group of genes.[4]

Not withstanding these important similarities, a number of differences also exist between the two degenerative diseases. The transmissible diseases are characterized by spongioform degeneration, a change which does not occur in Alzheimer's disease. Paired helical filaments, the morphological subunit of Alzheimer neurofibrillary degeneration, do not occur in the unconventional transmissible encephalopathies[5,6] and the amino acid composition of the amyloid of the neurtic plaques differs. The sequence of the N-terminal 15 amino acids of Alzheimer amyloid reported by Glenner and Wong[7] and Masters et al[8] does not resemble the amino acid sequences of scrapie amyloid as published by Prusiner et al[9] or Chesebro et al.[10]

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We have reported a change in chromatin structure which may affect gene expression in the neocortex of terminal Alzheimer's disease.[11,12] The changes in chromatin conformation are unique to Alzheimer's disease compared to other dementia associated conditions such as multi-infarct dementia, Lewy body encephalopathy, progressive supranuclear palsy of Steele, Richardson and Olszewski, Pick's disease and certain nosologically unclassified severe cerebral atrophic processes.[13] The changes in chromatin structure were detected by examining the digestion kinetics of chromatin by the enzyme micrococcal nuclease and examining the linker histones associated with dinucleosomes released during digestion. In view of the hypothesis that scrapie may affect gene expression in a manner similar to that which occurs in Alzheimer's disease, we have investigated chromatin structure in two strains of scrapie affected mice to assess further the possible similarities in these neurodegenerative conditions.

Experimental Methods

Scrapie Affected Tissue

Presymptomatic and symptomatic scrapie injected C57BL/6J mice were used. A 1% brain homogenate prepared from mice clinically affected with the 139A scrapie strain was injected intracerebrally. Control C57BL/6J and SJL/J mice were injected with 1% homogenate of normal mouse (C57BL/6J) brain. For the experiments using Swiss Albino mice, the scrapie strain was designated SSBP/1 Chandler strain, which had been passed serially through 18 sheep, 8 goats and two A.R.C. Swiss mouse passages prior to use. Control Swiss Albino mice had been injected with 1% normal mouse brain homogenate prepared from the same strain of mouse.

Alzheimer Affected Tissue

Brain tissues were obtained from The Canadian Brain Tissue Bank (Banting Institute, Toronto). Human control and Alzheimer affected brains were bisected in the sagittal plane and one half fixed in formalin and the other frozen at −90 degrees Centigrade. On the basis of clinical history and extensive histopathological examination the brains were divided into two groups: (1) an Alzheimer group with brains containing widespread senile plaques and tangles, obtained from patients with a history consistent with a diagnosis of Alzheimer's disease and (2) a Control group which had no clinical history of intellectual impairment or neurological disease. This latter group exhibited none of the histopathological markers for Alzheimer's disease.

Biohazard Containment

The isolation of Alzheimer and scrapie nuclei and all of the subsequent experiments involving these tissues were carried out under the conditions of MRC Level II (NIH P2) biohazard containment.[14] Scrapie infectivity in samples generated during the experiments was inactivated by one of the following means: decontamination with 6.0% sodium hypochlorite, autoclaving at 125°C for 120 minutes or incineration.

Biochemical Procedures

Preparation of Nuclei

Mixed nuclei from both neurons and glia of frozen (−70°C), unfixed control and scrapie infected whole mouse brain and control human and Alzheimer affected cerebral cortex were isolated in sucrose solutions by methods previously reported.[12] Using identical methods, nuclei from both control and scrapie infected whole brains of CJ57BL/6J and Swiss Albino mice were also prepared from fresh tissue.

Nuclease Digestions

A suspension of mixed nuclei was exposed to micrococcal nuclease (Worthington Enzymes, 1 U/ug DNA) for 10-12 minutes and the products of digestion were recovered and analyzed on an integrated 3.5% Tris-Borate-Ethylenediamine Tetraacetic Acid (EDTA)/18% acrylamide Tris-Glycine-Sodium Dodecyl Sulfate (SDS) gel system as described by Lewis et al.[12] DNA was assayed according to the methods of Burton[15] or the Hoechst 33258 assay.[16] Protein concentration was assayed by the BioRad method[17] using previously purified H1 and H1° linker histone as standards. Phenylmethylsulfonylfluoride (PMSF) at 1.0 mM was employed throughout the isolation procedures as a serine protease inhibitor. The ratio of linker histones to the core histone H4 was estimated by means of photodensitometer scans (Canalco Model G scanning densitometer) of Coomassie Brillant Blue stained gels. Validation of the quantitative analysis of the ratio of linker histones to the core histone H4 is described elsewhere.[12,13]

Salt Induced Histone Release

To examine the binding of linker histone to DNA, 100 ul aliquots of the 10 mM Tris-HCl, 0.25 M sucrose, 1.5 mM calcium chloride, 1.0 mM PMSF, pH 7.3 buffer containing 150 ug of isolated nuclei were centrifuged at 13.5 Kgav for 3 minutes. The supernatant was removed and the pelleted nuclei were exposed for 2 hours at 4°C to 100 ul of a NaCl solution ranging in concentration from 0.10 M to 0.75 M NaCl with repeated vortexing. The salt released linker histones were collected and analyzed on an SDS slab gel system as described by Crapper McLachlan et al.[13] The amount of linker histone is each extraction was quantitated by comparing values to the maximum amount extractable, i.e. the quantity extracted with salt concentrations of 0.75 M sodium chloride.

Results

Digestion Kinetics

Micrococcal nuclease is a compact globular ribo- and deoxyribonuclease of 17,000 dalton molecular weight which digests preferentially, but not exclusively, the linker regions of DNA between nucleosomes.[18] Figure 1A illustrates the separation of the products of digestion into mono, di and trinucleosome fractions on an ethidium bromide stained 3.5% Tris-Borate EDTA gel. Under standard conditions, as shown in Table 1, control mouse brain demonstrated 12.1% acid soluble nucleotide released after 10 minutes of digestion. For control human brain of average age 72 years, 12.9% acid soluble nucleotide was released. Nuclei of scrapie affected forebrain demonstrated reduced accessibility to DNA by the enzyme and only 9.8% acid soluble nucleotide was released after 10 minutes. Nuclei from Alzheimer affected neocortex, mean age 79 years, also demonstrated reduced accessibility, yielding the same numerical value of 9.8% solubility (Table 1).

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Dinucleosome Protein Content

The protein profiles of dinucleosomes released by micrococcal nuclease digestion of nuclei extracted from both control and scrapie affected mouse brain are shown in Figure 1. Three bands were usually observed in the H1 region on 18% tris-glycine SDS polyacrylamide gel of apparent molecular weights of approximately 30.8, 28.9 and 26.6 K daltons. These proteins are denoted as H1, H1° and H1°° respectively.[12] While the amount of histone H4 appears to remain equivalent in each type of dinucleosome isolated, there appears to be a quantitative increase in the H1 linker region of scrapie affected chromatin, (Figure 1C). For three strains of control mice ranging in age from 107 to 300 days, the relative ratios for H1, H1° and H1°° to H4 were 0.75, 0.38, 0.11 respectively, Table 2. At 107 days the scrapie injected C57BL/6J mice demonstrated no neurological signs but the ratios had risen to 1.00, and 0.65, for H1 and H1° respectively. No change in H1°° was noted. In the latter stages of the encephalopathy when neurological signs of scrapie were present, about day 160, both the C57BL/6J and the Swiss albino strains demonstrated an increase in the release of a population of dinucleosomes with elevated content of linker histones. For the C57BL/6J strain, the 28% increase noted in H1 in the pre-symptomatic animals rose to 51% in the symptomatic animals and H1° rose from 51% to 76%. The ratio of linker histone H1°° to H4 was not affected in either strain during the clinical phase of scrapie.

In comparison to the ratios of linker to core histones released from human neocortex, each of the linker histones was present in lower amounts. However, in Alzheimer's disease, the linker histones H1° and H1°° were markedly increased whereas H1 was not significantly different from control, Table 3. Interestingly, the single case of Pick's disease and mixed case of Pick's and Alzheimer's disease demonstrated an increase in both H1 and H1°.

Salt Extraction of Histones

One estimate of the affinity of binding of histones to DNA may be obtained by measuring the percentage protein released as a function of increasing ambient sodium chloride concentration in vitro. No difference was observed between age matched control and scrapie affected brain in the sodium chloride elution pattern for any of the histones, Table 4. In contrast, Alzheimer affected brain demonstrated marked increase in histone affinity for DNA.[13]

Table 1: Kinetics of Digestion of Control, Scrapie and Alzheimer Affected Chromatin

Tissue	Mean Age	B	N	% A.S.N.
Control mouse	160 da	86	3	12.1
Scrapie mouse	160 da	86	3	9.8
Control human	72 yr	18	12	12.9
Alzheimer human	79 yr	13	8	9.8

Chromatin were digested for 10 minutes under standard conditions as described in methods. B = number of brains, N = number of assays, % A.S.N. = per cent acid soluble nucleotides released during digestion. For mouse brain, B refers to number of brains pooled for each assay. For human brains, assays were performed on both individual brains and pooled nuclei from a maximum of 2 brains.

Table 2: Dinucleosome Protein Profile of Whole Mouse Brain, Control vs Scrapie

Strain	N	Age (days)	H1	H1°	H1°°	H1 Group
CONTROL						
C57BL/6J	2	107	0.78	0.43	0.13	1.34
C57BL/6J	2	160	0.81	0.46	0.06	1.33
Swiss albino	4	163	0.75	0.31	0.09	1.15
SJL/J	3	300	0.66	0.34	0.16	1.15
Mean		—	0.75	0.38	0.11	1.24
PRESYMPTOMATIC						
C57BL/6J	2	107	1.00	0.65	0.06	1.71
SYMPTOMATIC						
C57BL/6J	2	160	1.22	0.81	0.07	2.10
Swiss albino	4	163	1.00	0.47	0.08	1.55
Mean		—	1.11	0.64	0.08	1.83
Change (%) (Symptomatic/Control)			+48	+68	—	+48

Histone abundance is expressed as the ratio of H1 and H1 subtypes to Histone H4 for each preparation. N = number of assays.

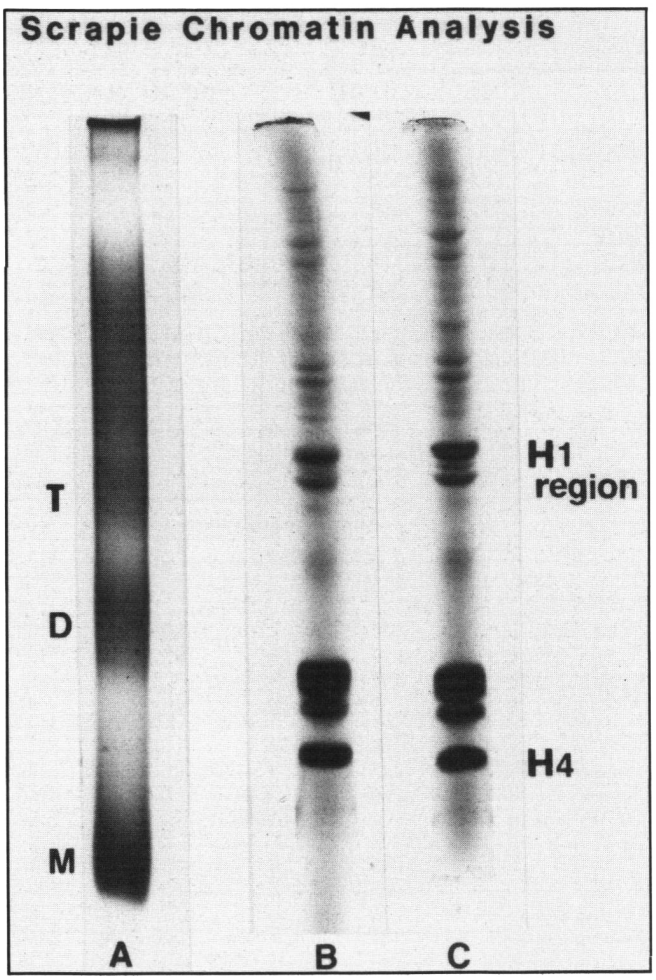

Figure 1 — SCRAPIE CHROMATIN ANALYSIS

Figure 1A - micrococcal nuclease digestion products of scrapie affected cerebral nuclei; T = trinucleosome, D = dinucleosome, M = mononucleosome.

Figures 1B and 1C - Dinucleosome protein profiles of respectively, control and scrapie affected chromatin. Note increase in scrapie H1 linker protein region.

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Discussion

Examination of the content of linker histone on dinucleosomes released by micrococcal nuclease fails to reveal an increase with normal aging in mouse brain, Table 2. At 300 days of age, the oldest mice available, SJL/J, exhibited the lowest ratios of linker histone to histone H4 of any of the strains of control mice. Therefore, the increase linker histone content of dinucleosomes released by micrococcal nuclease in two strains of mice infected with scrapie agent is most probably related to the infection rather than aging of the host.

The cellular mechanisms by which scrapie induces a progressive encephalopathy are unknown. The results of this study indicate that scrapie induces a change in chromatin conformation which renders DNA less accessible to enzymatic digestion by micrococcal nuclease. This change is associated with an increase in the amount of H1 and H1° linker histone associated with dinucleosomes released during digestion. The scrapie induced changes are progressive and precede the onset of neurological signs of encephalopathy, Table 2. This suggests that the changes in chromatin conformation play an active role in the pathogenesis of encephalopathy and are not simply the result of terminal stages of the disease process. One interpretation of these results is that the transmissible agent of scrapie alters gene expression in the host through a change in chromatin conformation. All linker histones apear to be involved in the formation of higher order chromatin structures.[19] The nature of the host genetic information affected in scrapie encephalopthy requires further investigation. Total polyadenylated RNA is not altered in scrapie affected brain.[20] Bountiff and Hunter[21] reported a change in low molecular weight RNA species in scrapie infected mice; one species increased (7S RNA) and one decreased slightly (8S RNA) compared to control mice. German et al[22] reported oligomers of 10 to 15 ribonucleotides in length to be increased in scrapie infected hamster brain. Low molecular weight RNAs are known to be involved in the generation of viable messenger RNA.[23] Whether the scrapie associated changes in low molecular weight RNA result from, or induce, chromatin conformational change is unknown.

Based on post mortem material obtained from the end stages of the illness, Alzheimer's disease is also associated with a remarkably similar change in chromatin conformation. While there is an overall increase in linker histones on dinucleosomes prepared from Alzheimer affected neocortex, the increase is due to an increase in the methionine containing H1° and a non-methionine containing histone H1°° rather than H1.[13] Indeed, the scrapie induced changes appear to resemble even more closely those found in Pick's disease in which H1 and H1° are increased (Table 3).[13] Alzheimer's disease is associated with a change in the saline elution pattern of histones from chromatin which was not observed in scrapie. The factors accounting for this putative increase in affinity of histones for DNA in the human disease are unknown but could involve a number of post-translational modifications such as phosphorylation, acetylation or metal ion binding. Recent work indicates that aluminum in vitro, in the range estimated to be 24 to 64 aluminum atoms per 200 base pairs alters the salt elution profile of linker histones to DNA of control human cerebral nuclei in a profile similar to that found in Alzheimer affected nuclei without added aluminum in vitro.[24,25] Finally, in contrast to scrapie, Alzheimer's disease is associated with a marked reduction in polyadenylated RNA.[26,27]

On balance, the differences between scrapie and Alzheimer's disease are of such a magnitude that scrapie does not appear to be a satisfactory model for the study of the molecular mechanisms underlying Alzheimer's disease.

Table 3: Dinucleosome Protein Profile of Whole Cerebral Cortex, Control vs. Alzheimer

	N	Age (yrs)	H1/H4	H1°/H4	H1°°/H4	H1 Group/H4
Control	7	72 (16)	0.44(.04)	0.18(.04)	0.08(.03)	0.71
Alzheimer	6	79 (7)	0.49(.21)	0.38(.17)	0.25(.11)	1.12
Picks	1	62 (0)	0.77	0.26	0.11	1.14
Picks + Alzheimer	1	62 (0)	0.66	0.24	0.27	1.17
Change (%) (Alzheimer/Control)			+11	+111	+213	+58

Histone abundance is expressed as the ratio of H1 and H1 Subtypes to Histone H4. Numbers in brackets are standard derivatives of the mean.

Table 4: Salt Elution of Histones from Control, Scrapie and Alzheimer Affected Chromatin

	Molar Concentration of NaCl						
	0.30	0.35	0.40	0.45	0.50	0.55	0.60
Histone H1							
Control mouse	8	14	18	48	80	90	100
Scrapie mouse	5	7	17	55	95	98	100
Control human	4	13	28	54	77	73	81
Alzheimer	0	0	0	14	48	79	82
Histone H1°							
Control mouse	15	22	23	43	62	80	98
Scrapie mouse	11	25	26	50	75	87	100
Control human	2	9	19	46	56	66	78
Alzheimer	0	0	0	8	45	67	75
Histone H1°°							
Control mouse	69	75	71	75	79	80	81
Scrapie mouse	93	100	100	99	97	99	100
Control human	5	19	32	57	57	66	76
Alzheimer	0	0	0	14	70	83	82

Histones extracted from nuclei at various NaCl concentrations and expressed as the percentage of maximum obtained at 0.75 M NaCl. Control human N = 7, Alzheimer human N = 6, Control mouse N = 2, Scrapie mouse N = 2; Scrapie from a pool of 100 whole mouse brains, (139A Scrapie agent) incubated in Swiss albino mice obtained from Animal Diseases Research Institute, Lethbridge, Alberta.

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