Use of H1 Histone to Test the Fidelity of Protein Biosynthesis in Mouse Tissues

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Proteins that do not contain certain amino acids can be used to study the fidelity of translation (or possibility of misincorporation) if these amino acids are introduced into systems in vitro or in vivo in labelled form. However, little work has been carried out by this approach (Loftfield & Vanderjagt, 1972; Edelman & Gallant, 1977; Buchanan & Stevens, 1978), because the problem of perfect purification of the relevant protein still remains difficult.

To test the fidelity of protein synthesis in vivo the H1 group of histones would be an obvious choice. The sequence analysis of histones (from calf thymus, rabbit thymus and some other sources) showed that histone H1 does not contain either methionine or cysteine. The solubility of histones in acids creates the unique possibility for their very high purification. Histone H1 does not participate in the nucleosome structure; it has higher molecular weight than H2A, H2B, H3 or H4 histones and can therefore be easily separated from them by different methods.

However, our first attempt to use [35S]methionine incorporation during histone-H1 synthesis in rat tissues for the study of mistranslation was unsuccessful. We found that all fractions of histone H1 from rat tissues are mixed with minor rat-specific methionine-containing subfractions (Medvedeva et al., 1975). This problem was not found for the case of chromatin from mouse tissues, where the main H1 histone was methionine-free, and a methionine-containing subfraction was present in tissue-specific histone-H1° fraction only (Medvedev et al., 1978).

The method for testing the error frequency was based on the comparison between the specific radioactivity of highly purified H1 histone and radioactivity of H2A, H3 and H4 histones (one methionine residue per molecule) or H2B histone (two methionine residues per molecule) some time after groups of CBA mice of different age received injections of [35S]methionine. In our experiments two groups of mice received double injection within 20h (10mCi/40 mice in each group). Isolation of nuclei from thymus, spleen and liver was the same as described in earlier work (Medvedev et al., 1977). H1 histones were extracted with 5% (w/v) HClO₄. Other histones were extracted by 0.2M-HCl from residue chromatin. Before these extractions non-histone proteins, which can contaminate H1 histone, had been eliminated by repeated extraction of chromatin with 0.35M-NaCl (Goodwin & Johns, 1973). Further purification of H1 histones and the separation of H1 and H1° fractions were made by Bio-Gel P-60 column chromatography. Although H1 and H1° histones from mouse liver and spleen chromatin appear as well-defined peaks during chromatography, histone H1 collected after the first fractionation is contaminated by small amount of H1° histone mixed with an H1° histone minor subfraction. To reach higher purification the combined fractions of the histone-H1 peak were dialysed, freeze-dried and used for repeated Bio-Gel column chromatography with an excess of unlabelled H1° histone separated earlier from liver chromatin of control mice.

Fig. 1 shows that the second Bio-Gel fractionation moved the remaining radioactivity from the histone-H1 into the histone H1° peak. The total radioactivity of combined fractions from the second Bio-Gel elution of H1 histone from about 40 spleens was only 21 c.p.m., whereas the radioactivity of a comparable amount of H2B histone was 21340 c.p.m. The ratio of these two values (1/1016) can be used for the approximate evaluation of the fidelity of translation. If the radioactivity of H1 histone represents misincorporation only, it could be assumed that a single histone H1 molecule per every 500 molecules has methionine (error frequency about 10⁻⁵ for each amino acid residue). However, the radioactivity in H1 histone is not only related to misincorporation. The failure to cleave the initiation methionine from the end of the polypeptide chain,
Fig. 1. Bio-Gel-fractionation profiles and radioactivity of histones extracted from spleen chromatin of 40 young (3-month-old) CBA mice

(a) Initial distribution of histone H1 and H1° fractions; (b) repeated fractionation of ten peak fractions of H1 histone (shaded area, the ten fractions contained 247 c.p.m.) (after dialysis and freeze-drying) with the excess of unlabelled histone H1° prepared from liver chromatin of control mice; the ten fractions for histone H1 and H1° contained 21 and 182 c.p.m. respectively; (c) the Bio-Gel distribution and radioactivity for H3, H2A and H2B histones (a different scale for measurement of activity was used); the ten fractions for histone H3 and H2A (together) and H2B contained 9820 and 21340 c.p.m. respectively. The ratios of radioactivity for the ten fractions were: H1/(H3+H2A), 467; H1/H2B, 1016. ○, Protein concentration (A230); ●, 35S radioactivity.

Ultra-minor contaminations by non-histone proteins or even somatic mutations could be mentioned among other possible causes of histone H1 radioactivity. These additional factors can be dissociated from misincorporation, but the new experiments with much higher [35S]methionine radioactivity or longer periods of administration are necessary to continue the purification procedures. The value of 10^-5 should be considered therefore not as the actual value, but as the highest possible limit of misincorporation. In our experiments it was different for different tissues: 2 x 10^-5
for histone H1 from liver chromatin; $5 \times 10^{-6}$ for histone H1 from thymus. Because H1 histone from mouse thymus has a higher turnover rate and does not contain a histone-H1$^\circ$ fraction (Medvedeva et al., 1978) a higher purity of H1 histone in this case could be expected.

We did not find an age-related difference in the ratios of specific radioactivities of histone H1 and other histones for liver chromatin. But the elution pattern of histone H1 radioactivity was different for two groups. In H1 histones from old animals the radioactivity and histone H1 concentration in the elution fractions were almost parallel, whereas in histone H1 from young animals the peak of radioactivity during Bio-Gel elution was related with the first fractions, where histone H1 concentration was low and non-histone contamination is more probable.

The maximal possible limit of errors shown in our work for H1 histone from mouse thymus ($5 \times 10^{-6}$) is lower than values from Loftfield & Vanderjagt (1972) for ovalbumin ($2 \times 10^{-5}$-$3 \times 10^{-5}$), Popp et al. (1975) for $\alpha$-globin ($3 \times 10^{-5}$-$6 \times 10^{-5}$) or Buchanan & Stevens (1978) for H1 histone from human fibroblast cultures ($7 \times 10^{-5}$ for young and $2 \times 10^{-4}$ for old cultures). It was, however, higher than the values quoted for Escherichia coli flagellin ($1.5 \times 10^{-6}$) (Edelman & Gallant, 1977).


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**Changes in the Relative Rates of Protein Synthesis and Breakdown during Muscle Growth and Atrophy**

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During muscle growth, protein synthesis must provide for net synthesis at a rate equal to the growth rate of each protein as well as for the replacement of protein degraded during the course of protein turnover. If the composition of the tissue is to remain reasonably constant, the growth component or net rate of protein synthesis will be the same for each protein. However, the replacement component of protein synthesis will vary according to the individual turnover rate, so that for each protein the growth component of synthesis will be a varying fraction of the overall rate. We might expect to find therefore a regulatory mechanism such that the synthesis of those proteins that turn over slowly will be increased to a greater extent during growth compared with those that turn over rapidly. Regulation during growth could also involve changes in the relative breakdown rates of proteins, since this would give added flexibility, particularly in situations in which the overall rate of breakdown is increased, such as during starvation-induced atrophy (e.g. Millward et al., 1976). If there are general control mechanisms such as these, then it would mean that the relative synthesis and breakdown rates of individual proteins could only unequivocally be defined in the steady state, and at all other times would vary according to the overall rates of synthesis and breakdown.

To investigate this problem we have made preliminary measurements of the relative synthesis and breakdown rates of the two main protein fractions in muscle,