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NUCLEAR BINDING OF EXOGENOUS HISTONES BY L CELLS AT LOW pH

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Procedures for the isolation of cell nuclei have frequently employed media of low pH, particularly citric acid solutions, for example 0.01 M citric acid, pH 2.6 (1–3). More recently, methods have been developed for the isolation of chromosomes which also make use of solutions of low pH, for example formate, pH 3.7 (4), and citrate, pH 2.1 (5), for more complete removal of cytoplasmic material.

Because of the use of these methods for studies of histone content and metabolism, we are prompted to describe here observations which show that, when cells are exposed to media of pH 3.5 or below, exogenous histones may be extensively bound to nuclei and chromosomes. This binding, which is a reversible process, may represent either an exchange of exogenous histone for cellular histone, or an additional binding above the in vivo level. Although we have not studied in detail the processes involved, we would like here to draw attention to the possibility that such binding of exogenous histones (or other basic proteins) could generate artefacts in analytical or metabolic studies of the histones of nuclei or chromosomes prepared in media of low pH.

METHODS

Fluorescein-labeled histone (f-histone) was prepared from calf thymus histone (Sigma Chemical Company, St. Louis, Mo., preparation H 101B-096) by standard methods (6), by labeling for 6 hr at an initial ratio of dye (fluorescein or rhodamine isothiocyanate) to protein of 0.5–2.0 μg/mg. The conjugates were extensively dialyzed against 0.01 N HCl and then 0.16 M NaCl, and passed through a column of Sephadex G25. They could be further precipitated in 90% ethanol from solutions in 0.1 N H_2SO_4 or 0.1 N NH_4OH, followed by redissolving at neutral pH, without change in their binding properties; but these properties were completely lost after digestion with crystalline trypsin (100 μg/ml, 15 hr, pH 7). Measurements of fluorescence and protein content of the conjugates showed a ratio dye/protein between 0.2 and 1.0 μg/mg.

Strain-L fibroblasts were grown to confluence on cover slips in Leighton tubes, with Eagle's medium (7) containing 10% horse serum. For investigation of the uptake of f-histone, the cover slips were rinsed five times in balanced salt solution (BSS) (8), then placed in a Petri dish, and covered with medium (without serum) containing f-histone; we omitted serum to avoid the possibility that serum proteins could compete, or complex, with histone. After incubation at 37°C for the desired time, the cover slips were rinsed five times in BSS, mounted in glycerol, and sealed for examination in the fluorescent microscope.

RESULTS

When L cells were incubated in medium containing f-histone (10–200 μg/ml) at pH 7 for periods of 1 hr or longer, fluorescence was observed only in a granular distribution in the cytoplasm (Fig. 1 *a*); nuclei and metaphase chromosomes showed no detectable labeling even after incubation for 15 hr. The same pattern of labeling was observed when the pH of the incubation medium was ad-

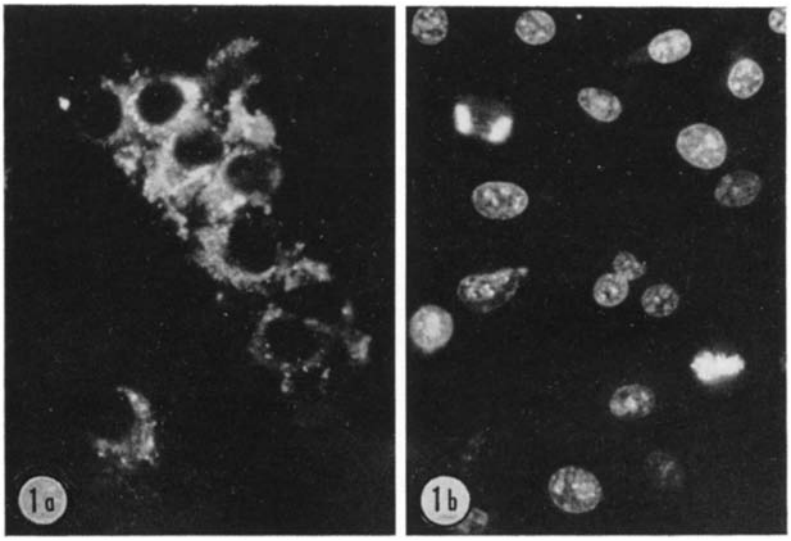

FIGURE 1 Patterns of uptake of fluorescein-labeled histone by L cells at pH 7 (*a*) and pH 3.5 (*b*). Incubation for 2 hr with histone at 100 μg/ml.

justed to values down to 4. The labeling pattern at lower pH values was, however, markedly different; nuclei and mitotic figures were now extensively labeled, but the cytoplasm was unlabeled (Fig. 1 *b*). Such labeling was seen in occasional cells at pH 4, and was general at pH 3.5 and below. Similar patterns of labeling were observed, under the same conditions, with HeLa and S180 cells.

The f-histone bound to nuclei and chromosomes at pH 3.5 could be removed by treatment of the labeled cells with trypsin (10 μg/ml in medium without serum, pH 7, 30 min); the binding of f-histone was depressed in the presence of an excess of unlabeled histone.

The histone bound at pH 3.5 is able to redissociate, and to exchange further with exogenous histone. Thus, when cells which had bound fluorescein-labeled histone were washed and transferred to medium without histone, the fluorescence was lost within 4 hr; if they were transferred to medium containing rhodamine-labeled histone, there was a progressive replacement of the initial yellow-green fluorescence by the red fluorescence of the rhodamine histone.

DISCUSSION

These experiments show that when cells of several common strains are incubated in medium of pH 3.5 or below, exogenous histones are able to bind to nuclei and chromosomes; this binding may occur to a small extent at pH values up to 4. The binding is reversible; the bound histones are released on further incubation in histone-free medium and are also able to undergo exchange with histones in the medium.

These observations suggest some limitations to the use of acid media for isolation of nuclei and chromosomes. Firstly, at pH values below 3.5, basic proteins originally in other locations in the cell may become bound to the chromatin. Secondly, since bound labeled histone is released in histone-free medium, and can undergo further exchange with histone in the medium, the initial binding of f-histone may itself represent an exchange with histone already bound to the DNA; there is thus the further possibility that histone constituents of the chromatin may be displaced by other basic proteins in the milieu, or simply released at pH's below 3.5. It thus appears preferable to avoid the use of media of pH below 3.5, and probably below pH 4, in the isolation of nuclei and chromosomes for studies of histone composition or metabolism.

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