

the 3 d in culture suggested reprogramming at the chromosome level and led us to look for corresponding changes in the nuclear proteins of these cells.

Tissue was isolated from the sub-apical ridge region of 370 stage 25 chick wing buds by transection of the limb tip 0.2–0.3 mm from the apex. No attempt was made surgically to remove the ectoderm which we estimate contributed less than 10% of total cells to the newly isolated material. Also, as sheets of ectoderm were lost during the early steps of nuclear isolation, the contribution of ectodermal nuclei to the final chromatin protein sample was probably a few per cent. Nuclei were isolated immediately from half the tissue, and from these chromatin was prepared and stored in a denaturing buffer containing phenylmethylsulphonyl fluoride, an inhibitor of proteolysis^{21,22}, at -20°C .

The other half of the tissue was cultured on 1.2% agar containing Ham's F-10 medium supplemented with 10% foetal calf serum and 1% bovine serum albumin. After 3 d in culture, nuclei were isolated from this tissue by the same procedure, chromatin was prepared, and the two preparations were run simultaneously on 25 cm 10% polyacrylamide gels containing 0.1% sodium dodecyl sulphate. The stained gels and the corresponding visible light spectrophotometer scans in Figs 1 and 2 were made from the preparation described, in which the freshly explanted and cultured material was taken from the same group of randomised embryonic limbs. Replicate analyses were done from separately prepared fresh and cultured tissue, yielding gels virtually identical to those pictured.

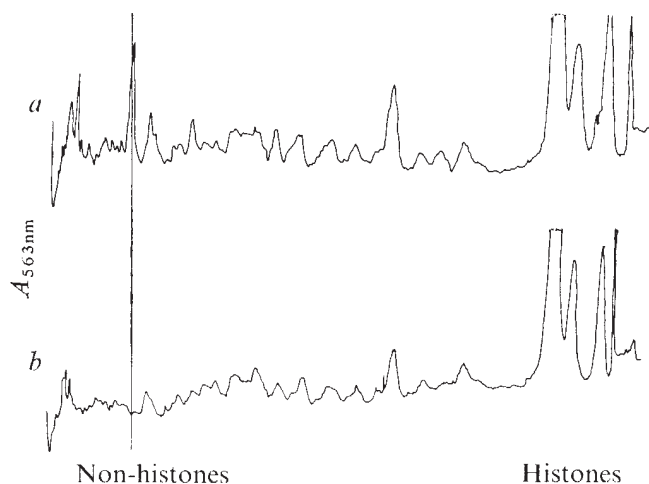


Fig. 2 Optical scans of the gels in Fig. 1 done at 563 nm on a Gilford spectrophotometer (a). Preparation from freshly explanted material. b, Preparation from cultured material. Tracing of prechondrocyte distinctive band is indicated by the line. The histone region may also contain some non-histone proteins.

In view of the phenotypic behaviour of the material analysed, we emphasise the following features of our electrophoretic data. First, we note the essential similarity between the two gels with respect to most of their stainable bands. While there are quantitative variations between some of the corresponding bands, this similarity appears greater than that usually found when different tissues or organs from a single individual or species are compared³⁻¹⁴. Second, the presence of a heavily staining band of low mobility (molecular weight between 125,000 and 150,000, estimated from markers in separate gels) in the freshly explanted, but not cultured material, is quite striking. This band is not present in similar gels of material prepared from cultured embryonic chick vertebral chond-

rocytes, fibroblasts or myogenic populations, though a low intensity band of similar mobility appears in chromatin preparations from primitive series chick erythrocytes (unpublished results of J.B., G.C.T.Y., and H. Holtzer). We do not exclude the possibility that the phenotypic differences between our freshly explanted and cultured material could be partly or entirely controlled by chromatin-associated molecules outside the limits of detection of our electrophoresis, staining, and scanning techniques. But we emphasise that our two preparations represented cell types which are adjacent in a developmental lineage and that the distinguishing protein (or class of proteins) was prominent against a relatively constant background, suggesting a role for this component in nuclear reprogramming. The all-or-none character of its disappearance (or loss of affinity for the stain) during the change from prechondrocyte to chondrocyte in culture recommends this system for *in vitro* studies of the mechanisms of cell differentiation.

We thank Dr H. Holtzer for facilities. This work was supported by grants from the National Institute of Health and the American Cancer Society to H. Holtzer. G.C.T.Y. is a recipient of a C.J. Martin Overseas Postdoctoral Fellowship from the National Health and Medical Research Council of Australia.

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Received November 3; accepted November 19, 1975.

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Expression of parental histone genes in the intergeneric hybrid *Triticale hexaploide*

Triticale hexaploide, the intergeneric hybrid resulting from the cross of diploid rye (*Secale cereale*) and tetraploid wheat (*Triticum durum*), offers a unique opportunity for the study of the expression of genes which are derived from two dissimilar organisms but exist together in the same cells. I have investigated the expression of histone genes in triticale because the histones are an extensively studied class of proteins with individual components which range from being highly conservative in an evolutionary sense to being probably species specific. For example, the sequence of histone F2a1 from peas and cows differs in only two of 102 amino acids¹, whereas different species of sea urchin have F1 histones of different electrophoretic mobilities². I

have found that in wheat, rye and triticale the evolutionarily conservative histones seem to be identical. There are, however, differences in the F1 histones of wheat and rye, and the gene for one of the wheat F1 histones is not expressed in the hybrid triticale.

Histones were extracted from dark-grown coleoptiles of triticale and from the rye and wheat parents as previously described³, and were analysed electrophoretically on short and long one-dimensional gels by the method of Panyim and Chalkley⁴ and on a new two-dimensional electrophoretic system.

Not surprisingly, considering the relatively close phylogenetic positions of wheat and rye, the highly evolutionarily conservative histones (F2a1 and F3) and the moderately conservative histones (F2b and F2a2) from all three organisms appeared identical on both one and two-dimensional gels (Figs 1 and 2). The only differences were in the F1 (lysine-rich) histones.

On short gels wheat F1 histones were resolved into four distinct bands, F1a, b, c and d. Long gels further resolved

wheat F1a into two electrophoretic bands, the slower of which I have called F1a'. This heterogeneity of wheat F1 histone is not due to phosphorylation since treatment of the isolated F1 fraction with alkaline phosphatase by the procedure of Sherod *et al.*⁷ did not affect the electrophoretic mobilities of the subfractions. Rye has only three F1 histones as resolved on both long and short gels. These have identical electrophoretic mobilities (as shown by electrophoresis of mixed samples of wheat and rye on long gels) to histones F1a, b and d (that is, rye histones are the same as wheat histones except that F1c and F1a' are lacking).

In the hybrid triticale the genes for histones F1a, b and d are expressed as is the gene for the wheat-specific F1a'. Even greatly overloaded gels, however, show no trace of the wheat-specific F1c histone.

The reason for the lack of expression of the wheat F1c gene is unknown, although it is not due to the loss of any whole chromosomes. Root tip squashes reveal the entire 2n complement of 28 chromosomes derived from wheat and 14 derived from rye, in the hybrid.

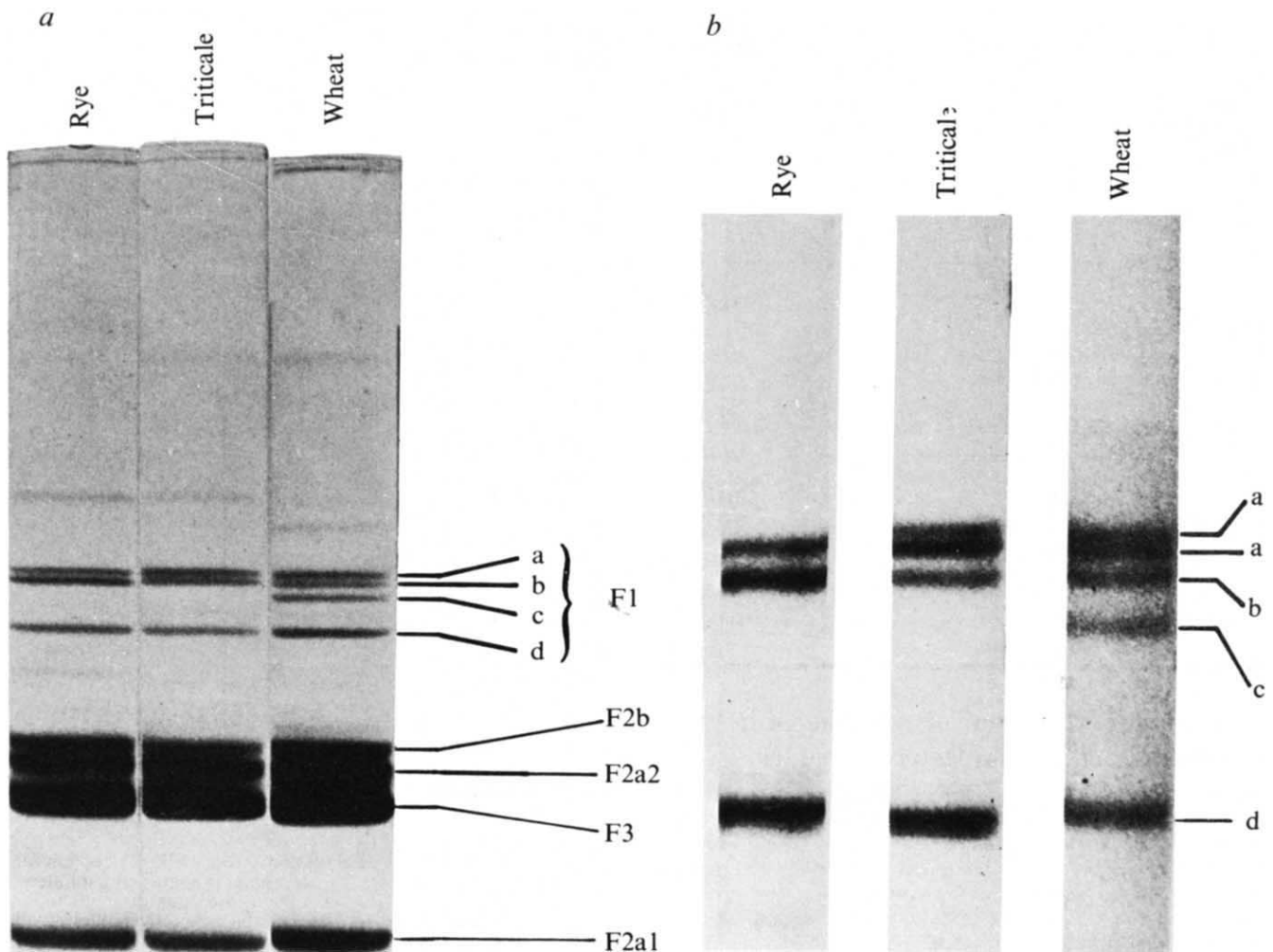


Fig. 1 a. Electrophoresis of wheat, rye and triticale whole histones on short gels by the method of Panyim and Chalkley⁴. The 10-cm gels were run for 4 h at room temperature, 2 mA per gel, then stained with amido black and destained by diffusion. All three species have histones F1a, b and d. The wheat-specific F1c is not expressed in the hybrid. The remaining histone fractions appear to be identical in all three plants. They have been named according to the nomenclature of Johns³ and identified by their fractionation properties, susceptibility to ferric chloride destaining and comparative mobility in one- and two-dimensional electrophoresis (manuscript in preparation). In this electrophoretic system the plant F2b and F2a2 histones have lower electrophoretic mobilities than histone F3 whereas histones F2b and F2a2 of mammals have greater electrophoretic mobility than histone F3 (ref. 3). **b.** Electrophoresis of wheat, rye and triticale F1 histones on long gels. The gels are of the same composition as the short gels in *a*, but are 20 cm long and were run at 0.75 mA per gel for 50 h at 2 °C. Other histone fractions have run off the gel, and only the portion of the gel containing the F1 histones is shown. Rye histones appear the same as in short gels but are separated further. F1a has been resolved into two bands in wheat and triticale, the slower of which has been labelled F1a'. The wheat-specific F1c is not expressed in the hybrid.

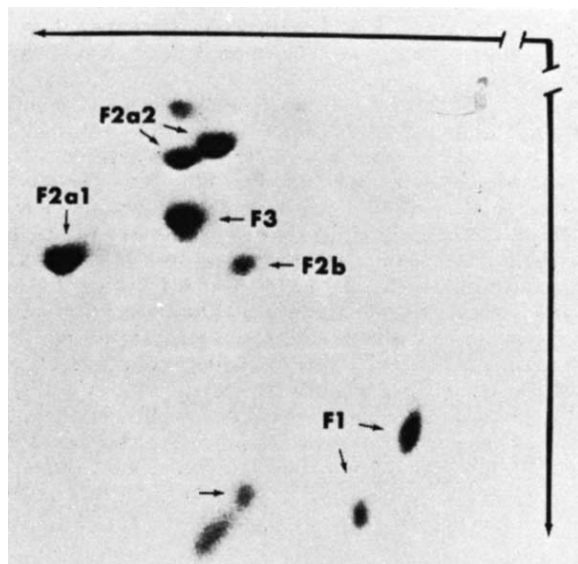


Fig. 2 Two-dimensional gel of triticale whole histone. Separation in the first dimension (right to left) was on acetic acid-urea gels according to Panyim and Chalkley⁴. The second dimension (top to bottom) was of the same composition but included 1% Triton X-100. The patterns for rye and wheat are identical except for the F1 histones. The rapidly moving, arrowed but unlabelled spots are probably histones which have not bound the Triton⁶.

This work was supported by grants from the Energy Research and Development Administration, the National Science Foundation and the National Cancer Institute.

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Transbilayer movement of cholesterol in dipalmitoyllecithin-cholesterol vesicles

THE movement of cholesterol across biological membranes is an important process for many cellular functions, but the mechanisms by which it occurs are not known. The movement of lipid molecules between the two halves of a bilayer, a process which has come to be known as flip flop, was first demonstrated to occur in films of stearate¹. Subsequently, several workers have reported flip-flop times for phospholipids in model systems, the values obtained varying between several hours and many days depending on the system studied and the techniques used²⁻⁵.

Lipid flip flop provides a possible mechanism for the translocation of cholesterol across membranes. To our knowledge only one study has been carried out to estimate the rate at which such a process could occur. Smith and Green⁶ reported a half time of 70 min at 30 °C for the flip flop of the fluorescent cholesterol analogue, sterophenol, in liposomes. We have carried out experiments to measure the flip flop of cholesterol itself in sonicated dipalmitoyllecithin-cholesterol vesicles, a

well known system⁷. On the basis of two kinds of experiments using different strategies, we conclude that cholesterol flip flop in this system is a very slow process if it occurs at all.

Lipid vesicles were made by sonication of dipalmitoyllecithin (DPL) (Sigma) and cholesterol in the mol ratio of 1:0.9 at 4 °C for 1 h under nitrogen, followed by centrifugation at 20,000g for 45 min at 4 °C. The purity of the DPL was checked by thin-layer chromatography before and after sonication, and there was no evidence of impurities or chemical degradation. Red-cell ghosts were prepared using the technique of Dodge⁸. The ghosts were used in excess as an acceptor membrane and no attempt was made to measure their leakiness. Red cells were labelled with ¹⁴C-cholesterol by incubating them in plasma containing ¹⁴C-cholesterol⁹. Lipids were extracted from red cells⁸ and from ghosts¹⁰, and cholesterol was determined by digitonin precipitation¹¹ and assayed by the method of Parekh and Jung¹².

In the first set of experiments DPL-cholesterol vesicles containing ³H-cholesterol and ¹⁴C-DPL were incubated at a cholesterol concentration of 10⁻⁵ M with red-cell ghosts at a cholesterol concentration of 4.2 × 10⁻⁴ M. This represented a 42-fold excess of ghost membranes, so that back exchange of the tritiated cholesterol would be insignificant. The ghosts and vesicles were incubated at pH 7.4 in a phosphate buffer (1.2 mM MgCl₂, 2.4 mM CaCl₂, 4.2 mM NaH₂PO₄, 1.7 mM Na₂HPO₄, 5.0 mM KCl, 13.5 mM Na₂CO₃, 117.8 mM NaCl, 10.0 mM glucose) at 37 °C. At various times aliquots were taken, the ghosts separated from the vesicles by centrifugation, and both the supernatant and pellet assayed for ³H and ¹⁴C. To prevent sticking of the vesicles to the ghosts, 10⁻⁶ M bovine serum albumin was added to the incubation medium. Figure 1a indicates that the exchange phenomenon saturates after 73% of the ³H-cholesterol has been removed from the vesicles, in ~ 24 h. No additional cholesterol could be removed from the

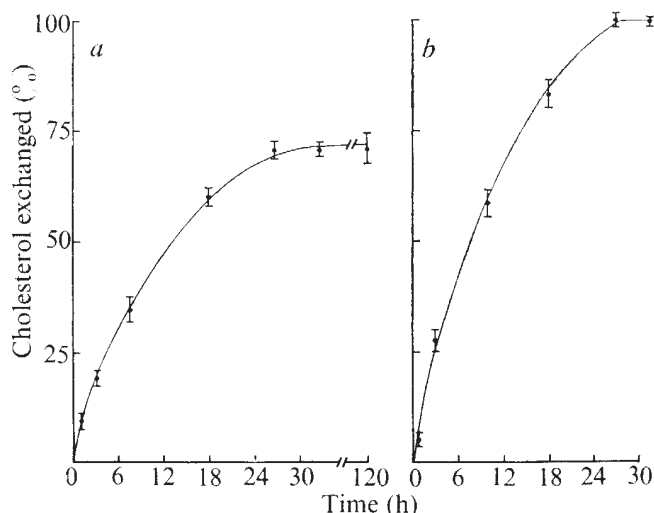


Fig. 1 *a*, Exchange of ³H-cholesterol from DPL-³H-cholesterol vesicles into erythrocyte ghosts where the labelled cholesterol was introduced into the original sonicated mixture. % cholesterol exchange refers to the amount of vesicle cholesterol transferred to the acceptor membrane. Correction (never exceeding 7%) was made for vesicles sticking to ghosts at each time point. *b*, Exchange of ³H-cholesterol from DPL-cholesterol vesicles into erythrocyte ghosts where the labelled cholesterol was introduced into the vesicles by a previous incubation with intact red cells containing ³H-cholesterol. The vesicles were stored at 37 °C for 48 h before being added to the ghosts preparation. The incubation medium contained streptomycin sulphate as an antibacterial agent and 10⁻⁶ M bovine serum albumin to reduce sticking of the vesicles to the ghosts. ¹⁴C-DPL was used as a control to monitor the degree of sticking and the concentration of phospholipid in the supernatant containing vesicles. The disappearance of labelled cholesterol from the vesicles corresponds with the appearance of label in the ghost fraction.