

TEMPLATE ACTIVITY OF UNFIXED METAPHASE CHROMOSOMES

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SUMMARY

Unfixed metaphase and non-metaphase cells were tested for their template activity with RNA polymerase. A device was used which disrupts the cell membrane by centrifugation, and which also ensures that the cells do not continue with their mitotic cycle during the transcription process. The template activity of acid/methanol fixed cells was also tested.

None of the unfixed metaphase cells transcribed RNA whereas most of the non-metaphase cells did. In contrast, using fixed cells both classes of cells were transcribed equally well and to a much greater extent. It was concluded that metaphase chromosomes *in vivo* cannot act as templates for RNA synthesis.

INTRODUCTION

During the cell cycle RNA synthesis is minimal in metaphase (King & Barnhisel, 1967). However, metaphase cells fixed on slides with methanol/acetic acid can be readily transcribed using exogenous RNA polymerase (Sederoff, Clynes, Poncz & Hachtel, 1973). This type of fixation removes most of the basic proteins which are attached to the chromosomes and which block the transcription of the DNA (Sumner, Evans & Buckland, 1973). Thus, *in vivo* it seems likely that metaphase chromosomes cannot function as templates because their DNA is blocked by inhibitory proteins. An alternative explanation is that metaphase cells have a low activity of RNA polymerase. The most direct way to test whether metaphase chromosomes can act as templates would be to isolate unfixed chromosomes and incubate them with polymerase and its substrates of radioactive nucleotide triphosphates. Chemically untreated chromosomes cannot be prepared since procedures for disrupting the cells usually involve extremes of pH or the use of detergents – which may also remove proteins from the chromosomes. An alternative method would be to measure the transcription in intact cells, but polymerase and triphosphates cannot permeate the cell membranes, and a further complication is that the cell can pass out of metaphase during the transcription process.

To overcome the difficulties we have developed a device which disrupts the cells with a minimum of treatment and permits the direct estimation of their template activity. Using this device we show that unfixed metaphase chromosomes are not in a state where they can be transcribed.

METHODS

Human FLAmnion cells (Flow Laboratories) were grown to half confluence in Roux bottles in Ham's F10 medium (Ham, 1963). The medium was poured off and 15 ml Dulbecco A salt solution (Dulbecco & Vogt, 1954) added and the bottle shaken for 20 s. The liquid was poured off and centrifuged at 1500 g for 5 min and the cell pellet was re-suspended in 0.5 ml Dulbecco's solution. The cell suspension contains 20-80% cells in metaphase. To swell the cells, 40 μ l of cell suspension were mixed with 0.4 ml 0.075 M KCl in the device described in Fig. 1 and kept chilled for 20 min. The device was centrifuged at 2500 g for 20 min in the M.S.E. bench centrifuge and the supernatant removed with a syringe leaving the cells flattened on the glass

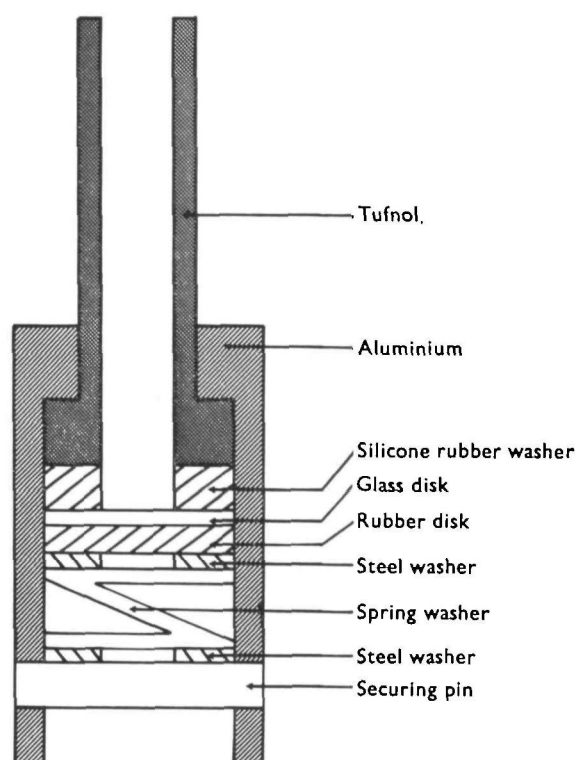


Fig. 1. Apparatus for spreading and incubating cells. The device was made of aluminium and Tufnol but can be made of other plastics. The maximum diameter is 1.6 cm, which will fit inside a flat-bottomed centrifuge tube holder. Glass disks were hand ground from microscope slides. The apparatus was assembled and compressed in a vice and the securing pin slotted in. Additional washers can be added next to the securing pin to obtain a watertight seal. A watertight seal is obtained when the lowest washer is level with the centre of the securing-pin hole before compression.

disk. This procedure disrupts cell membrane sufficiently to allow the polymerase and triphosphates to reach the chromatin template (see Fig. 2A). If cells were to be fixed, 0.4 ml of a 3:1 methanol:acetic acid mixture was added and the cells respun for 5 min, the supernatant removed and the cells refixed and allowed to dry. Four disks were prepared in this way and 4 others were prepared without fixation; 40 μ l of the transcription mixture were added. This was 0.1 M Tris HCl buffer pH 7.9 at 30°C, 2 mM spermidine, 2.5 mM $MnCl_2$, 1 mM ATP and contained 20 μ g bovine serum albumin, 1.2 μ Ci each of [5- 3H]-GTP (specific activity 1.7 Ci/mM), CTP

(1.8 Ci/mm), UTP (1.2 Ci/mm) and where indicated 4 μ g of RNA polymerase (357 units/mg, supplied by Boehringer).

The 8 devices were stacked in pairs and spun at 2500 g at 35°C for 20 min in the 4 x 50 ml swing out rotor of the Sorvall R2 centrifuge; 0.35 ml 12.5 mM GTP, UTP, CTP and 0.075 M KCl were added and the tubes spun at 2500 g for 2 min in the bench centrifuge and the supernatant removed. The cells were then washed in the same way with 0.075 M KCl, twice with methanol/acetic acid fixative and dried. The glass disks were removed and washed successively in 5% trichloroacetic acid, twice in fixative and dried. The cells were examined with phase-contrast microscopy for quality then glued to microscope slides with dePex, dried, and dipped in Kodak NTB3 nuclear emulsion. After exposure for 19 days, the slides were developed and stained with 25% Giemsa for 20 min.

RESULTS AND DISCUSSION

Photographs of transcribed fixed and unfixed cells are shown in Fig. 2. The most informative disk was of the transcription of unfixed cells in the presence of exogenous polymerase (Fig. 2A). Photographs were taken of several random fields and the cells were classified as metaphase or non-metaphase and scored arbitrarily for significant labelling above background for the field. Of 100 metaphase cells scored none were labelled and of 100 non-metaphase 65% were labelled. The reasons for 35% being unlabelled could be because they are dead cells, cells which have their DNA in the wrong state for template activity or cells which were not properly disrupted so that the polymerase and triphosphates failed to reach the chromatin. In the same way it is possible that the metaphase cell has no grains because the cell membranes do not disrupt properly and are therefore less permeable to the polymerase and triphosphates. This explanation is unlikely because many of the metaphase cells scored showed chromosomes which were very widely spread and clearly separated from the rest of the cell, and thus would definitely be in contact with the polymerase and triphosphates. None of these chromosomes had any grains. Furthermore, during the swelling process in 0.075 M KCl, if the time of swelling is prolonged many of the metaphase cells spontaneously disrupt which would suggest that their cell membranes are more fragile than that of the interphase cell. In the absence of exogenous polymerase, unfixed cells showed very little labelling above background (Fig. 2B) which shows that the amount of RNA synthesized by the endogenous polymerase is very low by comparison in these experiments.

In contrast, virtually all of the fixed metaphase and non-metaphase cells were all equally transcribed by the exogenous polymerase and were very heavily labelled in comparison with the unfixed cells (Fig. 2C). If the fixed cell is assumed to have all of its DNA available for transcription then this result agrees with the report by Davidson & Hough (1969) who estimate only a very small amount (0.28%) of the DNA in the vertebrate cell is transcribed *in vivo*.

It is not the purpose of this report to speculate why the metaphase chromosome is inactive, but it is unlikely to be due to the simple contraction of the DNA on a macro scale, since fixed metaphase and non-metaphase cells are transcribed equally well. Fixation has been shown to remove basic proteins which block the DNA (Sumner *et al.* 1973) and this result, taken with the report that polylysine added to fixed cells

reduces their template activity (Marfey & Li, 1974), would suggest that the basic proteins in the metaphase cells are acting as the main inhibitors of template activity.

These results show by a direct method that the DNA in metaphase chromosomes cannot be transcribed.

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Fig. 2. Autoradiographs of cells transcribed in the fixed and unfixed state in the presence and absence of exogenous polymerase. *I*, interphase cell; *M*, metaphase cell. $\times 750$. *A*, unfixed + polymerase. Metaphase cells with few grains and interphase cells with many grains. *B*, unfixed - polymerase. Very little labelling over any cells without polymerase. *C*, fixed + polymerase. Very heavy labelling in relation to unfixed cells over both metaphase and interphase cells. *D*, fixed - polymerase. In absence of polymerase the labelling is very much reduced and grains are probably due to non-specific binding of the triphosphates.

