MEMBRANE ASSOCIATED DNA ("M-BAND") IN GROWING AND CONFLUENT 3T3 CELLS

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1. Introduction

3T3 mouse fibroplasts stop growing after reaching a confluent monolayer [1]. In these "contact inhibited" cultures, most of the cells are arrested in G_1 of the cell cycle with no DNA-synthesis going on [2]. There are several indications in the literature that replicating DNA is associated with the nuclear membrane [3–5], suggesting that the association of DNA with the nuclear membrane may be altered after the cells reach confluency.

In this paper we report preliminary studies which indicate that the proportion of membrane associated DNA is significantly lower in confluent 3T3 cells than in growing cells. To investigate the association of the bulk of DNA with nuclear membranes, the "M-Band" technique was used, in which membrane associated DNA is coprecipitated with magnesium-lauroyl-sarcosinate to a complex of higher buoyant density, whereas "free" DNA is not [5, 6].

2. Methods

2.1. *Cell culture and radioactive label*

Exponentially growing 3T3 cells (8×10^3 cells/cm^2) were labelled during 30 hr with 1 μCi/2×10^5 cells [^{3}H]thymidine (26 Ci/mmole) respectively 1 μCi/8×10^5 cells [^{14}C]thymidine (56 mCi/mmole). Cultures were trypsinized and cells were plated at saturation density (1.2×10^5 cells/cm^2) in dishes overlaid with coverslides and held in contact for further 30 hr. ^{14}C-prelabelled cultures were stopped by removing the medium, washed with Hanks solution and kept frozen at −20° until further procedure. ^{3}H-prelabelled cultures were trypsinized again and disseminated at lower density (8×10^3 cells/cm^2). At various times one of these growing cultures was stopped and frozen in the manner described above.

2.2. *M-Band technique*

One coverslide with confluent cells was added to each growing culture, and the two were processed simultaneously using the M-band technique [5, 6]: After adding 1 ml of a Mg and Ca free buffer (0.9% NaCl, 0.01 M Tris Cl, pH 7.5) cells were lysed by very gently mixing with 0.1 ml of a 1% Sarcosyl solution (sodium-*N*-lauroyl-sarcosinate, Schuchardt) and allowed to stand in ice for 3 min. After the addition of 0.01 ml of 1 M $MgCl_2$, the Mg-Sarcosyl crystals formed within 10 min. 0.5 ml of the mixture was layered on to the top of 11 ml of a 15–42% (w/v) linear sucrose gradient (0.1 M KCl, 0.01 M $MgCl_2$, 0.01 M Tris-HCl, pH 7.5) and the Mg-Sarcosyl–membrane complex was banded in the middle of the gradient by centrifuging 20 min at 40 000 *g* (SB 283, IEC B60, 4°).

2.3. *Radioactivity assay*

Radioactivity of the gradient fractions (0.6 ml, ISCO) were assayed by a modified [7] Mans and Novelli filter paper method. Two 0.1 ml aliquots of each fraction were pipetted on to filter discs (Whatman), extracted 3 times in 5% TCA and once in 5% acetic acid. Double label was counted in 5 ml toluene scintillator in a Packard 3380 Tri-carb.

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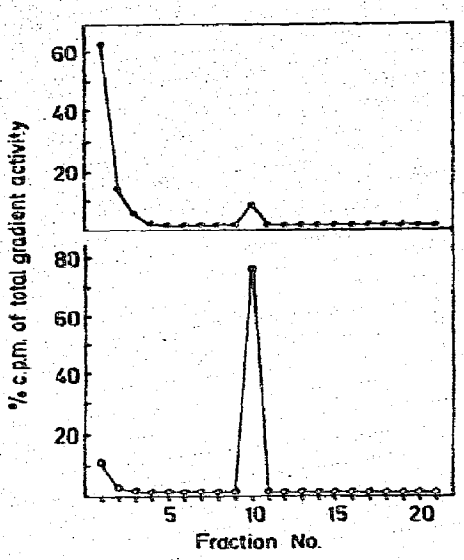

Fig. 1. Distribution of Mg-Sarcosyl bound and "free" DNA from growing and confluent 3T3 cells by density equilibrium centrifugation in a sucrose gradient. 3T3 cells were incubated with radioactive thymidine for more than 4 S-phase periods to get generally labelled DNA. These labelled cells were then seeded at high concentrations (1.2×10^5 cells/cm^2) and allowed to reach confluency and thereafter partially seeded again at low cell densities, permitting cells to enter S-phase. M-bands were prepared from these two culture types. ^{3}H-resp. ^{14}C-cpm of each gradient were summed up and the part of each fraction is plotted in % cpm. (●—●—●) [^{14}C]Thymidine prelabelled DNA of the confluent culture, (○—○—○) [^{3}H]thymidine prelabelled DNA of the growing culture, 12 hr after release from the confluent state.

3. Results and discussion

As shown in fig. 1, 76% of the total DNA is associated with the M-band when growing cells were analysed, with only 12% found in the case of confluent cells. In each experiment growing and confluent cultures were lysed and analysed simultaneously to keep shearing of DNA comparable in both cases.

Less than 5% of the confluent cells took up Trypan blue indicating that the low percentage of DNA in the M-band is not due to dead cells. As a further control, in one experiment the double labelling was reversed, with [^{14}C]thymidine being given to growing and [^{3}H]thymidine to confluent 3T3 cells. The results of this experiment corresponded exactly to those of the standard procedure.

Table 1
Relative amounts of membrane associated DNA ("M-Band") from confluent and growing 3T3 cells.

		Prelabelled DNA in the M-band fraction (%)
Confluent 3T3 cells		7–15
Growing 3T3 cells	0.5	76
(hr after release	3	70
from confluency)	6	68
	12	76
	16	64

Cells were labelled, seeded and "M-Band" assays were carried out according to Methods. Growing cells are those at indicated times after release from confluent state. Of the total activity of the whole gradient only the percentage of label found in the M-band fraction is given.

Immediately after seeding (0.5 hr) at low cell densities, the ratio of M-band radioactivity to total radioactivity reaches the value found for growing cells (table 1). These data indicate that DNA may be associated with the nuclear membrane without DNA replicating going on. This suggests that cells in G_1 which are ready to go into S-phase differ from confluent (G_0?) cells by their higher degree of DNA–membrane association. Polysome analyses had revealed earlier that confluent cells show a higher ratio of bound to free polysomes than growing cells [8], another indication that the membrane status of confluent (G_0?) cells may fundamentally differ from that obtaining during any stage of the cell cycle of growing cells.

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