On the Filamentous Elements in the Nucleoli of Chick Embryo Fibroblasts

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With one plate (fig. 1)

Summary

Filamentous inclusions (= nucleolonemata) in the nucleoli of chick embryo fibroblasts and their behaviour during the cellular cycle are described. Their number is about the same as that of the macrochromosomes. They originate in anaphase as thin threads along the chromosomes. During the reconstitution of the daughter nuclei they aggregate within two nucleoli. They disintegrate with the prophase of the next mitosis.

Introduction

FILAMENTOUS inclusions have been found in the nucleoli of a variety of cells, with the light microscope (Estable & Sotelo, 1951, 1952; Lettré & Siebs, 1954; Denues & Mottram, 1955; Horstmann & Knoop, 1957) and with the electron microscope (Borysko & Bang, 1951; Bernhard & others, 1952 *a*, *b*; Porter, 1954; Yasuzumi & others, 1958). Because of their apparently different dimensions and also because of the different techniques applied, it has been questioned whether all these structures are homologous (Stich, 1956). In some instances no nucleolar inclusions were found at all (Rodkiewicz, 1959). It has been suggested (Serra, 1958, Rodkiewicz, 1959) that the filamentous inclusions found in nucleoli might be artefacts, caused by fixation or by the heterogeneous impregnation of a homogeneous ground substance. Unfortunately, even where these elements have been demonstrated during life, artifacts might have been caused by the methods employed (Hughes, 1952; Denues & Mottram, 1955).

A filamentous structure has been found with the electron microscope in chicken fibroblasts (Borysko & Bang, 1951). However, no individual filaments could be distinguished. Hughes (1952) found that the nucleoli of chicken fibroblasts can disintegrate into smaller fragments, which might or might not have existed during life as preformed elements. Lettré and Siebs (1954) claimed that the filaments found in the nucleoli of chicken fibroblasts are parts of chromosomes and that they give a positive Feulgen reaction.

Since the existence of filamentous nucleolar inclusions and their behaviour during the cellular cycle might be of great theoretical interest (Vincent, 1955), we have examined fibroblasts from chicken embryos with regard to these elements and to their formation after mitosis.

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Methods

The cells were grown in hanging drop cultures, containing equal volumes of 25% chick embryo extract and chicken plasma. Vigorously growing cultures were fixed in 10% formaldehyde which had been neutralized with calcium carbonate. After thorough rinsing they were impregnated with silver nitrate according to a method of Cajal (Romeis, 1928). The impregnated preparations were examined under phase contrast.

Results and Discussion

At the periphery of the cultures, the cells thinned out to such an extent that even the nucleoli were somewhat flattened, and the nucleolar inclusions,

Table 1

Number of nucleoli per nucleus	*Predominant shape of the nucleolar inclusions*	*Number of nucleolar inclusions*	
		low estimate	*high estimate*
2	granular	9	15
2	filamentous	14	18
2	filamentous	12	17
2	filamentous	10	13
2	granular	9	17
2	filamentous	12	15
2	filamentous	10	18
2	filamentous	12	13
2	filamentous	9	16
1	filamentous	15	19

impregnated with silver nitrate, could be distinguished. In small, globular nucleoli they had the shape of granules. In nucleoli that were flattened the inclusions appeared mostly as nodular filaments (fig. 1, A, B). Since their thickness approaches the limit of resolution of the light microscope, it was not always possible to identify each single filament within a group with certainty, and their exact number could not be determined. Table 1 shows numbers of filaments and granules counted in several nuclei at the periphery of the culture. Two counts are given for each nucleus. The low count was derived by interpreting any group of nucleolar inclusions which was not clearly resolved in favour of the lower number, and the higher count was obtained by doing the opposite. It is worth noting that the numbers counted are about the same as that of the macrochromosomes (12, according to Brink (1959)).

We found no evidence that the nucleolonemata persist through mitosis. They disintegrate during prophase. In anaphase (fig. 2, A), a thin line appears along each chromosome. These lines are impregnated in the same way as the

Fig. 1 (plate). A, nucleolonemata in a flattened nucleolus.
B, drawing of the same nucleolus, to show the position and the number of the filaments.

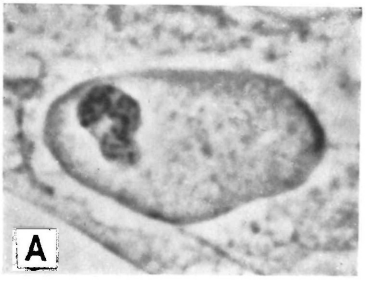

FIG. 1

E. GUTTES *and* S. GUTTES

nucleolar inclusions. From here on the transition to the nucleolar inclusions in the daughter nuclei is continuous. In the daughter nuclei (fig. 2 B), while the individual chromosomes can no longer be distinguished, these threads

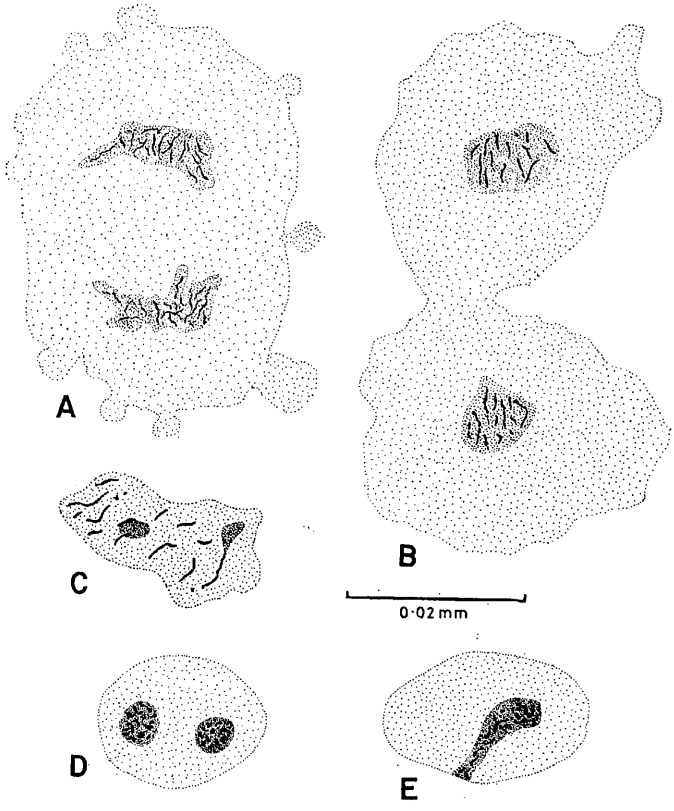

FIG. 2. A, anaphase; nucleolonemata reappearing along the chromosomes. B, daughter nuclei. C, daughter nucleus. Two small nucleoli; most of the nucleolonemata still in the extra-nucleolar parts of the nucleus. D, E, nucleolonemata within two (D) and one (E) nucleolus.

shorten and become thicker. The reappearing nucleoli (fig. 2, C) first contain only a few inclusions imbedded in the *pars amorpha*, while most of the short rods and filaments are still in the extra-nucleolar parts of the nucleus. In the final stage of the reconstitution of the nucleus, all the nucleolonemata are aggregated in either one or two nucleoli (fig. 2, D, E), and no similarly

impregnated elements are found in the rest of the nucleus any more. We found no evidence that any inclusion of the nucleoli gave a positive Feulgen reaction.

The continuous transition from the impregnated lines along the chromosomes, first to the short filaments and granules within the daughter nuclei, and then to an intermediate stage where some of the elements are found outside and some inside the nucleoli, until finally they are only found within the nucleoli, does not favour the argument that they are mere artefacts produced by fixation or heterogeneous impregnation of a homogeneous ground substance.

It is conceivable that the nucleolonema is produced by the dissociation of the chromosomes at anaphase into two complementary halves of which the one, containing the DNA, is to be transmitted through the generations, while the other has only a temporary existence through the next interphase.

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