THE RELATIONSHIP OF RIBOSOMAL RNA SYNTHESIS TO THE FORMATION OF SEGREGATED NUCLEOLI AND NUCLEOLUS-LIKE BODIES

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ABSTRACT

The relationship of ribosomal RNA (rRNA) synthesis to nucleolar ultrastructure was studied in partial nucleolar mutants of *Xenopus laevis*. These mutations are the result of a partial deletion of rRNA genes and therefore allow studies on nucleolar structure and function without using drugs that inhibit rRNA synthesis. Ultrastructural studies demonstrated that normal embryos have reticulated nucleoli that are composed of a loose meshwork of granules and fibrils and a typical nucleolonema. In contrast, partial nucleolar mutants in which rRNA synthesis is reduced to less than 50% of the normal rate have compact nucleoli and nucleolus-like bodies. The compact nucleoli contain granules and fibrils, but they are segregated into distinct regions, and a nucleolonema is never seen. Since other species of RNA are synthesized normally by partial nucleolar mutants, these results demonstrate that nucleolar segregation is related specifically to a reduction in rRNA synthesis. The nucleolus-like bodies are composed mainly of fibrils, and the number of such bodies present in the different nucleolar mutants is inversely related to the relative rate of rRNA synthesis. Although the partial nucleolar organizers produce segregated nucleoli in these mutants, they organize morphologically normal, but smaller, nucleoli in heterozygous embryos. Alternative explanations to account for these results are discussed.

In comparison with other genes, the ribosomal RNA genes are unique in that their transcription occurs in a complex chromosomal organelle – the nucleolus. The fine structure and size of the nucleolus are very sensitive to changes in ribosomal RNA (rRNA) synthesis (16, 30, 31). The nucleolus therefore serves as a cytological indicator of the transcription of rRNA genes and, in this respect, is analogous to the puffs of polytene chromosomes. The nucleolus is much more complex than a puff, however, since it is also the cellular site for ribosomal subunit production which involves the interaction of a large number of RNA and protein components not synthesized in the nucleolus. The complexity of this process makes it very difficult, at the molecular level, to interpret nucleolar structural changes that occur normally or in response to various drugs. It seems likely that the maintenance of normal nucleolar morphology would require the normal transcription of the rRNA genes as well as a balance among rRNA synthesis, the production and accumulation of ribosomal and nucleolar-specific proteins, and the transport of completed ribosomal subunits out of the nucleolus.

The partial nucleolar mutants of *Xenopus laevis*

offer a unique opportunity to study nucleolar structure and function without using drugs that inhibit rRNA synthesis. These mutations are due to a partial deletion of the nucleolar organizer which results in a reduction of rRNA synthesis and the production of a small nucleolus (16, 21–23). The partial nucleolar organizer p^{l-1} contains about 200 rRNA genes which represents only one-half the number present in a normal (+) nucleolar organizer. The *l-1* designation indicates that this mutation is lethal in the hemizygous condition and is the first partial nucleolar mutant we described. Embryos with at least one complete nucleolar organizer (+/+, $+/p^{l-1}$, and $+/o$) are phenotypically normal and synthesize rRNA at the same rate. Embryos carrying only the partial nucleolar organizer (p^{l-1}/o embryos) live only 9–12 days and synthesize rRNA at about 25% of the normal rate. In this report we will show that the p^{l-1} nucleolar organizer produces a segregated nucleolus in p^{l-1}/o embryos but a morphologically normal, though smaller, nucleolus in $+/p^{l-1}$ embryos. We will also describe a new nonviable nucleolar mutant (p^{l-3}/p^{l-4}) and show that it synthesizes rRNA at 50% of the normal rate and has segregated nucleoli throughout its limited life-span. Some of these results were presented at the Annual Meeting of the American Society for Cell Biology (10, 20).

MATERIALS AND METHODS

Extraction and Characterization of RNA

Details of the labeling, RNA extraction, and polyacrylamide gel electrophoresis techniques have been described previously (19). Embryos were micro-injected with a solution of [5,6-^{3}H]uridine (39.3 Ci/mM) and [8-^{3}H]guanosine (19 Ci/mM) at 20 mCi/ml and incubated for 2 or 24 h in Barth's saline (1). After determining the nucleolar phenotype of the labeled embryos, RNA was extracted from single +/+ or p^{l-3}/p^{l-4} embryos using the cold phenol technique (3) or the chloroform-phenol technique (27). In the latter technique, the embryos were homogenized in 2 ml of NETS buffer (0.1 M NaCl, 0.01 M EDTA, 0.01 M Tris, pH 7.4, 1% sodium dodecyl sulfate [SDS]) and extracted with an equal volume of chloroform-phenol (1:1). After centrifugation to separate the aqueous and organic phases, the organic phase and interphase were re-extracted twice with 2 ml NETS. The aqueous phases were combined and re-extracted with chloroform-phenol until the interphase disappeared. The RNA was precipitated with 2 vol ethanol and analyzed on 2.7% polyacrylamide gels cross-linked with ethylene diacrylate.

Electron Microscopy

Embryos at various stages of development were cut in half and the endoderm removed. The pieces were fixed in 2.5% glutaraldehyde, 0.1 M phosphate buffer, pH 7.2, for 2–4 h at room temperature and then overnight at 4°C. The embryos were washed three times with 0.1 M phosphate buffer containing 0.2 M sucrose and then, in most cases, postfixed in 1% osmium tetroxide in the same buffer for 1 h. After routine ethanol and propylene oxide treatment, the tissues were embedded in a mixture of Araldite 502 and Epon 812 (24). Ultrathin sections were double stained in 3% aqueous uranyl acetate followed by lead citrate (28). Electron micrographs were made in an Hitachi HU-11F electron microscope at 75 kV.

RESULTS

The Origin of the p^{l-3} and p^{l-4} Mutations

The progeny of phenotypically normal frogs from the wild population usually have two equal-sized nucleoli in all binucleolated cells. Miller and Gurdon (21) have shown, however, that the progeny of normal frogs may include embryos with unequal-sized nucleoli. The $+/p^{l-3}$ and $+/p^{l-4}$ frogs used in this study were found among the progeny of two normal adults obtained from a local supplier (NASCO, Fort Atkinson, Wis.). The nucleolar condition of 84 progeny of these two normal frogs was determined by examining a small piece of tail tissue of each embryo by phase-contrast microscopy. Of the embryos examined, 57 had two equal-sized nucleoli (+/+ embryos) and 27 had one large nucleolus and one very small nucleolus in most cells ($+/p$ embryos). Some of the $+/p$ embryos were raised to maturity and mated with their siblings. In all of these matings, as described below for the $+/p^{l-3}$ and $+/p^{l-4}$ heterozygotes, the progeny included a class of embryos (p^l/p^l) which died at the swimming tadpole stage of development. All of the $+/p$ mutants we have tested carry recessive lethal mutations but we do not know whether they are all identical. The data below have been obtained from studies of the progeny of two of these heterozygous mutants ($+/p^{l-3}$ and $+/p^{l-4}$). We have not determined the number of rRNA genes carried by the p^{l-3} and p^{l-4} nucleolar organizers.

Cytological Characteristics and Development of the p^{l-3}/p^{l-4} Mutant

The progeny of the mating between $+/p^{l-3}$ and

$+/p^{l-4}$ heterozygotes included three types of embryos which could be distinguished by their nucleolar condition. Of 74 embryos examined at stage 26 (25), 29 had two equal-sized nucleoli (+/+), 34 had two unequal nucleoli ($+/p^l$), and 11 had two small nucleoli plus nucleolus-like bodies (NLB) in some cells (p^{l-3}/p^{l-4}). It was not possible to distinguish $+/p^{l-3}$ and $+/p^{l-4}$ embryos, and hereafter they will be referred to as $+/p^l$ embryos. The p^{l-3}/p^{l-4} mutants had two nucleoli in most cells, but in contrast to +/+ embryos their nucleoli were smaller, more spherical, and appeared to have a higher density when examined by phase-contrast microscopy. Furthermore, the p^{l-3}/p^{l-4} embryos had some cells with one or two small NLB, a situation we never encountered in +/+ or $+/p^l$ embryos.

The +/+ and $+/p^l$ embryos develop normally whereas the p^{l-3}/p^{l-4} embryos die as swimming tadpoles at stage 42. The p^{l-3}/p^{l-4} embryos are phenotypically indistinguishable from their siblings until stage 40, but they can be identified by their nucleolar condition soon after gastrulation. At stage 40, the p^{l-3}/p^{l-4} embryos became edematous and microcephalic as described previously for p^{l-1}/o and o/o embryos (21, 36). It is of interest that at a given stage both the cytological and phenotypic abnormalities of the p^{l-3}/p^{l-4} embryos are less pronounced than those of p^{l-1}/o or o/o embryos.

Ribosomal RNA Synthesis in Normal and Mutant Embryos

To determine the relative rate of rRNA synthesis of p^{l-3}/p^{l-4} mutants, embryos at stage 35 or 40 were labeled by micro-injection of a mixture of [^{3}H]uridine and [^{3}H]guanosine. 2 or 24 h after injection, total RNA was extracted from +/+ and p^{l-3}/p^{l-4} embryos using cold phenol or chloroform-phenol and was analyzed on 2.7% polyacrylamide gels. In most cases the analysis of rRNA was made using single embryos. This allowed a comparison of the relative rates of rRNA synthesis in embryos with similar or different nucleolar phenotypes and eliminated the possibility of contaminating a sample of embryos through an error in the identification of nucleolar condition.

The relative amount of radioactivity in 4S, 18S, and 28S RNA was determined from RNA radioactivity profiles such as those shown in Fig. 1; the results of several experiments are presented in Table I. The exact amount of radioactivity injected into each embryo was not determined (usually between 20–100 nl). To eliminate the resulting variability in rRNA radioactivity per embryo, we have calculated the relative rate of rRNA synthesis from the amount of radioactivity in 18S and 28S RNA compared to the amount in total RNA or 4S RNA. There does remain, however, some variation in the relative amount of radioactivity in rRNA of embryos belonging to the same nucleolar class which we are unable to explain (Table I). In a previous publication we used the percentage of the total RNA radioactivity in 18S and 28S RNA as a measure of the relative rate of rRNA synthesis in p^{l-1}/o embryos (16). By this method, the rate of rRNA synthesis in p^{l-3}/p^{l-4} embryos was calculated to be 40 or 50% of the rate attained by +/+ embryos after a 2- or 24-h labeling period, respectively. When the radioactivity in 4S RNA was used as the standard, the relative rate of rRNA synthesis in p^{l-3}/p^{l-4} embryos was calculated to be about 25% of the normal rate after a 2- or 24-h labeling period. Since we have not measured the absolute rates of synthesis of rRNA and 4S RNA, it is difficult to determine which of these calculations is more accurate. Nevertheless, the results presented in Table I clearly show that the p^{l-3}/p^{l-4} mutants synthesize 18S and 28S RNA at less than half the normal rate. Evidence has been presented previously which indicates that the reduction in the relative amount of radioactivity in rRNA of partial nucleolar mutants reflects a reduced rate of rRNA synthesis rather than changes in the size or specific activity of the RNA precursor pools (16).

The results in Table I also demonstrate that the relative percentage of total RNA radioactivity in 18S and 28S RNA is much lower when the chloroform-phenol technique, rather than the cold phenol technique, is used to extract the RNA. The decrease in the relative amount of rRNA in chloroform-phenol extracted samples is probably due to the recovery of a greater amount of non-rRNA with this technique (27). As expected, however, the calculation of the rate of rRNA synthesis in p^{l-3}/p^{l-4} embryos relative to +/+ embryos is not affected by the RNA extraction procedure.

Ultrastructural Observations on Normal and Mutant Embryos

The fine structure of nucleoli of normal and

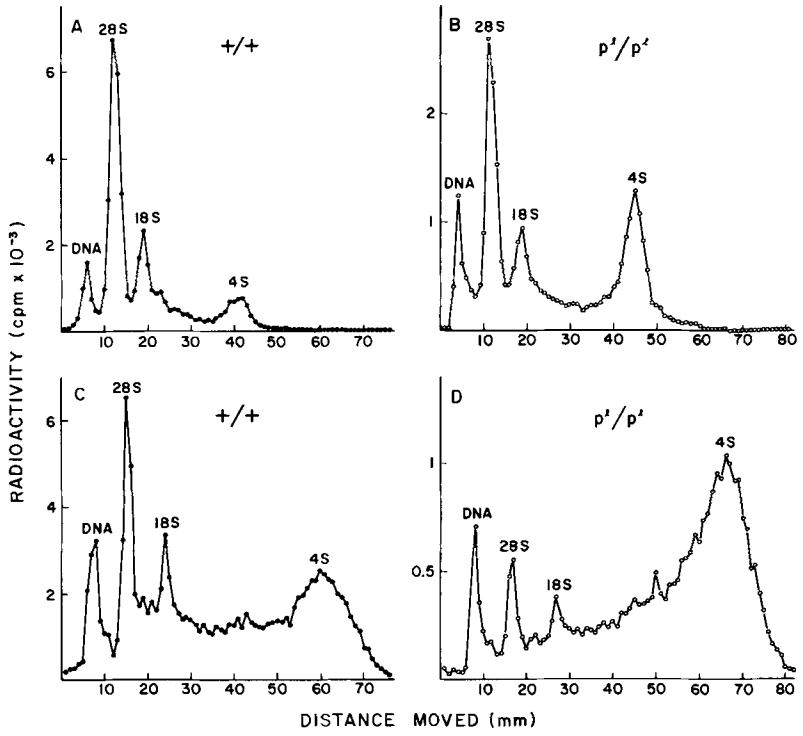

FIGURE 1 RNA synthesis in normal and mutant embryos. Embryos from a cross of two $+/p^l$ frogs were injected with a mixture of [5,6-^{3}H]uridine and [8-^{3}H]guanosine. After 2 or 24 h, total RNA was isolated from single embryos and analyzed on 2.7% polyacrylamide gels. (A and B) RNA from +/+ (A) and $p^{l\text{-}3}/p^{l\text{-}4}$ (B) embryos labeled 24 h during stages 35–40. (C and D) RNA from +/+ (C) and $p^{l\text{-}3}/p^{l\text{-}4}$ (D) embryos labeled 2 h during stage 40.

TABLE I

The Relative Amount of Radioactivity in Ribosomal and 4S RNA of Normal and Mutant Embryos

Nucleolar genotype	Hours labeled	Total RNA Radioactivity	RNA radioactivity	
			18S + 28S	4S
		cpm	*%*	
RNA isolated with cold phenol				
+/+	24	28,917	40.4	6.6
+/+	24	19,567	44.8	11.1
+/+	24	43,688	47.7	6.9
+/+*	24	210,972	57.2	9.4
+/+	2	83,458	38.7	5.7
$p^{l\text{-}3}/p^{l\text{-}4}$	24	23,456	26.0	15.3
$p^{l\text{-}3}/p^{l\text{-}4}$	24	27,464	28.0	18.9
$p^{l\text{-}3}/p^{l\text{-}4}$	24	35,548	27.6	11.3
$p^{l\text{-}3}/p^{l\text{-}4}$	24	29,763	17.4	20.2
$p^{l\text{-}3}/p^{l\text{-}4}$	2	34,677	16.7	10.6
RNA isolated with chloroform-phenol				
+/+	2	113,687	13.9	19.8
+/+	2	91,668	13.2	23.7
$p^{l\text{-}3}/p^{l\text{-}4}$	2	25,512	4.9	35.6
$p^{l\text{-}3}/p^{l\text{-}4}$	2	28,917	5.0	36.7

* Results from a sample of 40 embryos. All other values in the table are from the analysis of RNA of single embryos. Correction was made for background radioactivity as described by Gurdon (11) before calculating the relative amount of radioactivity in the 18S, 28S, and 4S RNA peaks.

mutant embryos from two different crosses was examined by electron microscopy after fixation in glutaraldehyde. Four different types of progeny (+/+, $+/p^{l-1}$, $+/o$, and p^{l-1}/o) are found in the $+/p^{l-1} \times +/o$ mating, and three types (+/+, $+/p^{l}$, and p^{l-3}/p^{l-4}) are found in the $+/p^{l-3} \times +/p^{l-4}$ cross. All seven types of embryos from the two different crosses have been analyzed at various stages of development. There are no detectable differences in the fine structure of nucleoli of wild-type embryos (+/+) from the two crosses. Likewise, a comparison of the nucleoli of heterozygous embryos from the two crosses did not reveal any differences in morphology. Consequently, the nucleolar morphology of these embryos will be described in the general categories for +/+ and $+/p^{l}$ embryos. The observations on p^{l-3}/p^{l-4} mutants and their siblings have been made on early tailbud embryos (stage 26) or swimming tadpoles (stage 42). The fine structure of the nucleoli of p^{l-1}/o mutants and their normal siblings was studied at stages 19, 24, 33/34, 40, and 47. The p^{l-1}/o embryos only develop to stage 42 but they remain alive for a further 6–9 days. After stage 42 the p^{l-1}/o embryos are staged according to the development of their normal siblings.

The nucleoli of +/+, $+/p^{l}$, and $+/o$ embryos have an irregular shape and consist of intermixed granules and fibrils (Fig. 2). The granules and fibrils are loosely arranged giving the normal nucleolus a reticulated structure with a typical nucleolonema. The fine structure of the nucleolus did not vary when two nucleoli or a single nucleolus were present in cells of +/+ embryos. Normal nucleoli have not been observed in p^{l-1}/o or p^{l-3}/p^{l-4} embryos at any of the developmental stages we studied. The nucleoli of these nonviable mutants contain granules and fibrils similar to those of normal nucleoli but they are always segregated into clearly defined regions (cf. Figs. 2 and 3). The fibrils and granules of the segregated nucleoli are tightly packed, and a nucleolonema is never seen. Although the partial nucleolar organizer produces a segregated nucleolus in p^{l-1}/o and p^{l-3}/p^{l-4} mutants, it organizes an ultrastructurally normal nucleolus in $+/p^{l}$ embryos (cf. Figs. 4 and 5). In p^{l-1}/o embryos a single segregated nucleolus is present in all cells whereas in p^{l-3}/p^{l-4} embryos two segregated nucleoli are often found (Fig. 5). The nuclei of p^{l-1}/o and p^{l-3}/p^{l-4} embryos also contain NLB in some cells. These structures are much smaller than the segregated nucleoli and are composed primarily of fibrils. In some instances, we have observed NLB with two distinct fibrillar regions which differ in their relative electron opacities (Fig. 6). Furthermore the fibrillar region of the segregated nucleolus is sometimes composed of similar light and dark fibrillar components suggesting a close structural relationship between it and the NLB.

DISCUSSION

In this report we have shown that two partial nucleolar mutants of *Xenopus*, p^{l-1}/o and p^{l-3}/p^{l-4}, are characterized by nuclei having segregated nucleoli and fibrillar NLB. However when the p^{l} nucleolar organizer shares a nucleus with a normal + organizer, as in $+/p^{l}$ embryos, it produces an ultrastructurally normal nucleolus and in this situation NLB are not found. We have also shown that the p^{l-3}/p^{l-4} mutants synthesize rRNA at less than 50% of the rate attained by +/+ and $+/p^{l}$ embryos. It was previously demonstrated that the p^{l-1}/o mutants have only 25% of the normal number of rRNA genes and synthesize rRNA at about 25% of the normal rate (16). Thus it is clear that a partial deletion of rRNA genes, which leads to a reduction of cellular rRNA synthesis, can result in the production of segregated nucleoli and fibrillar NLB.

NLB were first described by McClintock (18), and their fine structure and possible origins have been discussed by Swift and Stevens (33). Ultrastructural and cytochemical evidence indicates a close structural relationship between the ribonucleoprotein fibrils of NLB and the fibrillar region of normal nucleoli (13, 33). In support of this relationship we have shown that both the NLB and the nucleoli of p^{l-1}/o mutants have similar segregated patches of light and dark fibrillar components. However, the macromolecular composition of NLB is probably quite different from that of the fibrillar region of the nucleolus. The fibrillar region of the nucleolus contains the rRNA genes with nascent rRNA precursors, ribosomal proteins, and some nonribosomal nucleolar proteins (4, 6, 30, 31). If the NLB contain components normally found in the fibrillar region of the nucleolus, these are likely to be nonribosomal nucleolar proteins since the anucleolate mutant of *Xenopus* which accumulates large numbers of NLB (13) does not accumulate rRNA precursors (2) or ribosomal proteins (12). Similarly, when rRNA synthesis is inhibited with actinomycin D or camptothecin, most nucleolar components continue to be synthesized, but only nonribosomal

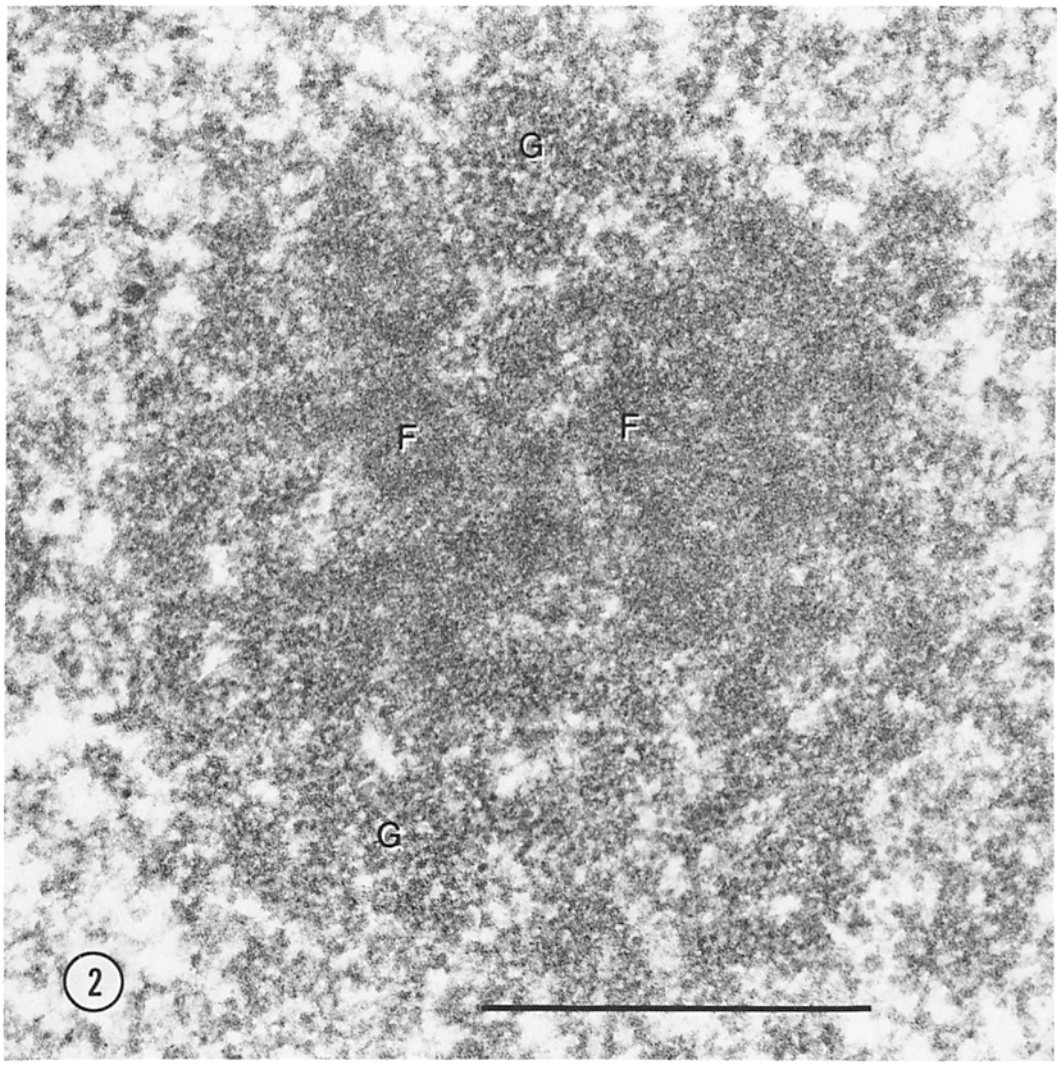

FIGURE 2 Electron micrograph of a wild-type nucleolus from a stage 26 $+/p^{l}$ embryo. Note the loose appearance and intermixture of fibrils (*F*) and granules (*G*). × 50,600. Bar = 1 μm.

nucleolar proteins accumulate in the nucleolus (5, 17, 32, 38). While it has not been shown directly that NLB contain nonribosomal nucleolar proteins, the biochemical and morphological evidence summarized above supports this view.

All nonviable nucleolar mutants of *Xenopus* have NLB: *o/o* embryos have one to eight NLB per nucleus (7), p^{l-1}/o embryos have one to four NLB in many cells (21), and only a few cells with one or two NLB are found in p^{l-3}/p^{l-4} embryos. Thus the number of NLB that accumulate in these mutants is inversely related to the relative rate of rRNA synthesis. Furthermore, it was noted by Fischberg and Wallace (8) that the relative amount of nucleolus-like material that accumulates in different tissues of anucleolate mutants (*o/o*) appears to be directly related to size of the nucleoli present in the same tissues of normal individuals. These results suggest that the components of the NLB continue to be made at a rate that is adjusted to meet the metabolic demand for ribosome production even though rRNA synthesis has been reduced or completely prevented.

Segregated nucleoli have been described previously in cells treated with drugs such as actinomycin D and during the terminal stages of cell differ-

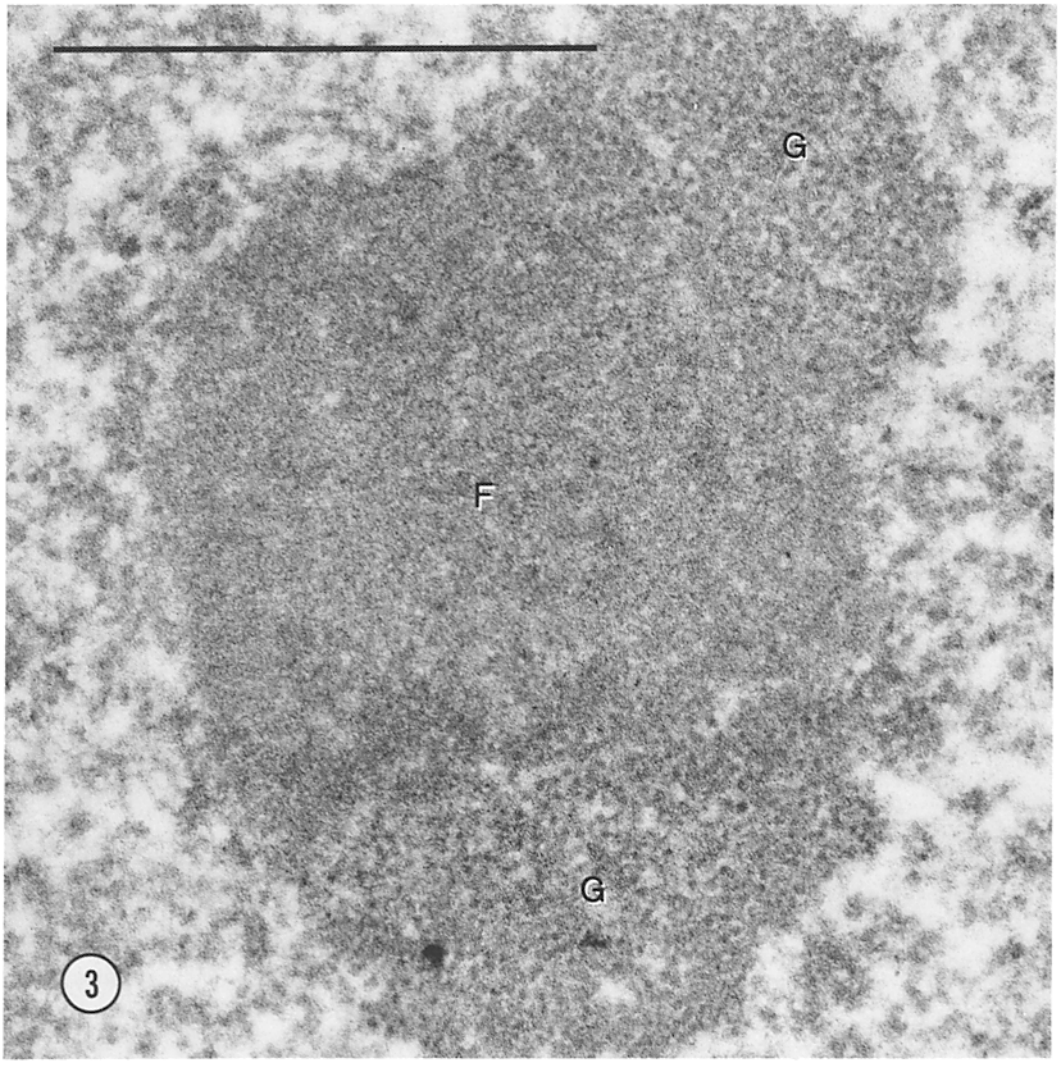

FIGURE 3 Electron micrograph of a segregated nucleolus from a stage 26 p^{l-3}/p^{l-4} embryo. Note the compact appearance and the segregated fibrils (*F*) and granules (*G*). × 70,400. Bar = 1 μm.

entiation of various tissues (30, 31). In these situations a morphologically normal nucleolus is converted to a segregated nucleolus, and this change is accompanied by a severe reduction in total RNA synthesis. It is commonly accepted that nucleolar segregation is due specifically to the inhibition of rRNA genes rather than to the other metabolic effects of actinomycin D (9, 30). Our findings strongly support this view, because in partial nucleolar mutants the remaining rRNA genes and other genes appear to function normally. The nucleolar mutants produce functional ribosomes, they do not accumulate rRNA precursors, and RNA species other than rRNA are synthesized normally (2, 16, 19, 20, 23). Furthermore, the segregated nucleoli are found in all cells during early development when the nucleolar mutants are phenotypically normal and cell division and differentiation are proceeding normally (21). Thus, the formation of segregated nucleoli in partial nucleolar mutants demonstrates that segregated nucleoli are a morphological defect related specifically to a reduction of rRNA synthesis.

Although all forms of segregated nucleoli are associated with a reduction of rRNA synthesis and share certain morphological characteristics, it is

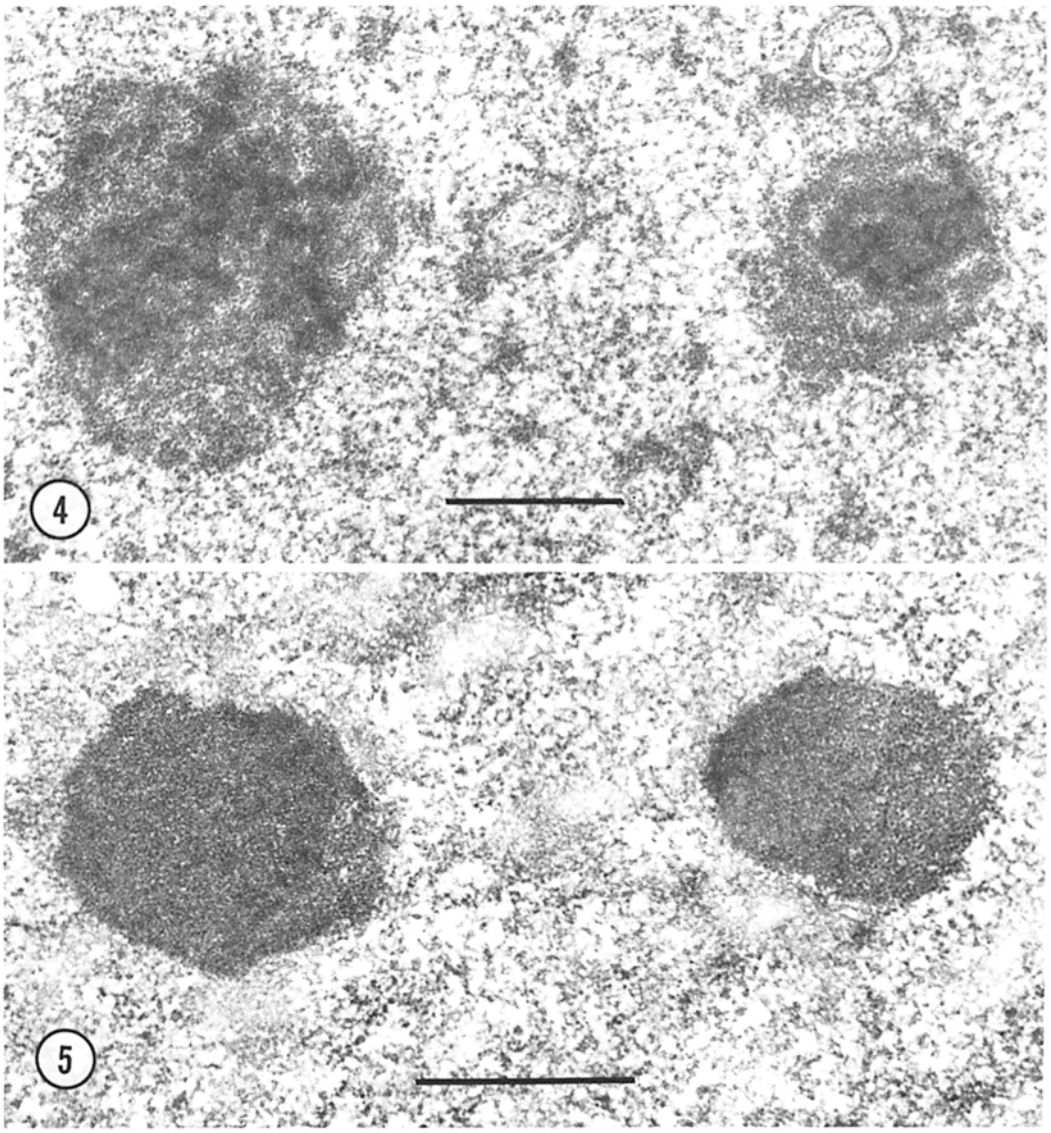

FIGURE 4 Electron micrograph of nucleoli from a stage 19 $+/p^{l}$ embryo. Note that the small nucleolus assumes the wild-type ultrastructural organization in $+/p^{l}$ embryos. × 23,700. Bar = 1 μm.

FIGURE 5 Electron micrograph of nucleoli from a stage 26 p^{l-3}/p^{l-4} embryo. Both nucleoli have a compacted and segregated appearance. × 28,600. Bar = 1 μm.

not known whether they are the result of a common molecular mechanism. It has been suggested that actinomycin-D-induced nucleolar segregation is due to the condensation of rRNA genes caused directly by the binding of actinomycin D (9, 30) or indirectly by the release of nascent rRNA precursors from their template (29). The reduction in rRNA synthesis in p^{l-1}/o mutants is due to a partial deletion of rRNA genes, suggesting that in these mutants rRNA gene condensation is not required for the formation of segregated nucleoli. We cannot rule out the possibility that the formation of segregated nucleoli is the result of condensation of nucleolar chromatin which does not include the rRNA genes. If condensation of nucleolar chromatin is responsible for

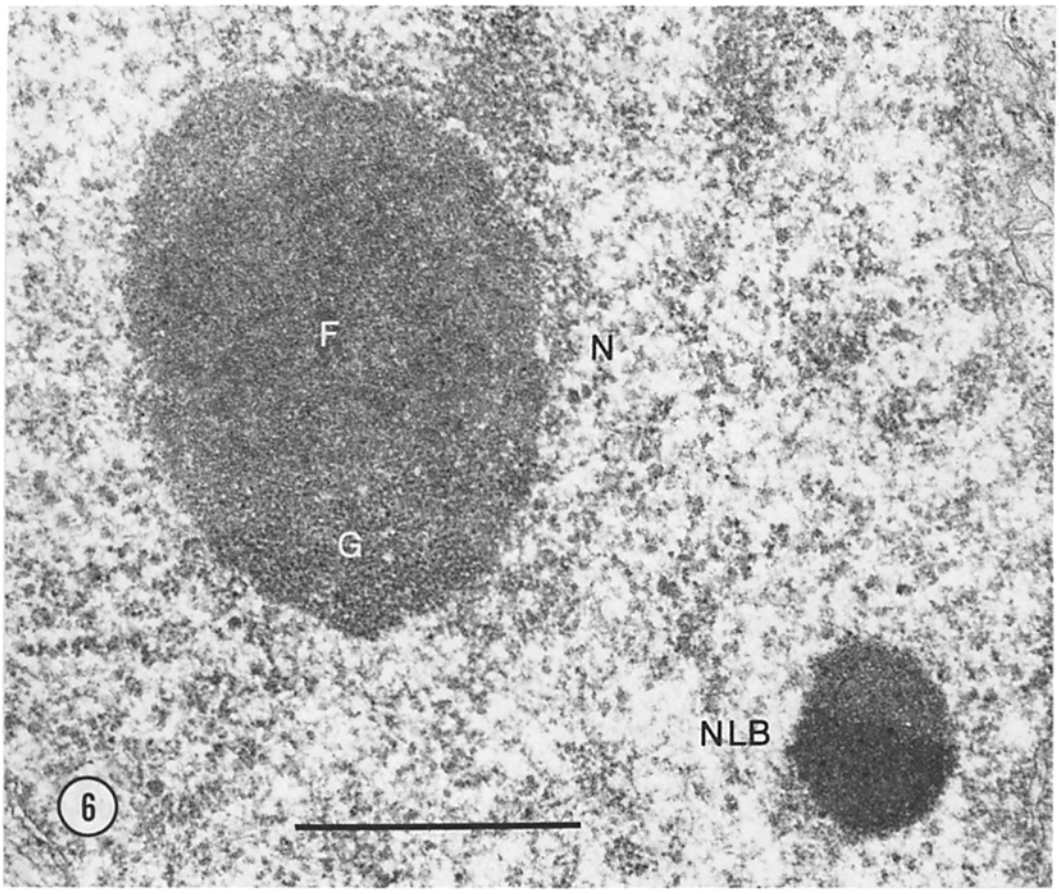

FIGURE 6 Electron micrograph of a nucleolus (*N*) and nucleolus-like body (*NLB*) from a stage 47 p^{l-1}/o embryo. Fibrillar (*F*) and granular (*G*) regions are compact and segregated in the nucleolus. The NLB is composed of light and dark fibrillar components which are segregated into discrete areas. × 37,400. Bar = 1 μm.

the segregation phenomenon in partial nucleolar mutants, a cyclic decondensation and condensation would have to be invoked in order to explain the reformation of segregated nucleoli after each cell division.

The segregated nucleoli of p^{l-1}/o and p^{l-3}/p^{l-4} embryos appear to be blocked at a stage of development between the compact, primarily fibrillar nucleolus typical of gastrula embryos (14, 15) and the reticulated nucleolus found in cells actively producing ribosomal subunits. Normally the conversion of a fibrillar nucleolus to a reticulated nucleolus is correlated with an increase in nucleolar size and rRNA synthesis. During this transition, the granules and fibrils become intermingled and loosely arranged forming a sponge-like network (26, 35). Any hypothesis proposed to explain this apparent blockage in nucleolar development must also account for the presence of NLB in partial nucleolar mutants and the finding that the p^l nucleolar organizer produces an ultrastructurally normal nucleolus in $+/p^l$ embryos. There are two explanations which may be suggested to account for these findings. The first is that a specific nucleolar component, or components, necessary for the formation of a reticulated nucleolus is missing in p^{l-1}/o and p^{l-3}/p^{l-4} mutants but is present in normal amounts in $+/p^l$ embryos. This explanation suggests that the missing component can be supplied by a + nucleolar organizer but not by a p^l organizer. Since the partial nucleolar mutants make functional ribosomes, the missing com-

ponent would be essential for the formation of a normal nucleolus but would not be required for ribosome production.

Alternatively, it may be proposed that all of the components required for the conversion of a fibrillar nucleolus to a reticulated nucleolus are present in normal and mutant embryos, but the reduction of rRNA synthesis in mutant embryos leads to an unbalanced supply of nucleolar components. Excess nucleolar components (nonribosomal nucleolar proteins?) would accumulate in NLB and at the site of the p^l nucleolar organizer, preventing the formation of a reticulated nucleolus. In $+/p^l$ embryos, which synthesize sufficient rRNA to meet the cellular demand for ribosome production, there is a balance between the production and use of nucleolar components, so that NLB do not form and the p^l nucleolar organizer produces a reticulated nucleolus. We do not have any direct evidence that excess fibrillar material accumulates at the p^l organizer and prevents the normal loosening and intermingling of nucleolar granules and fibrils. Nevertheless, the ribonucleoprotein fibrils of NLB are capable of forming dense nuclear aggregates and, as described above, they may be precursors to the fibrillar region of the segregated nucleolus which presumably contains the p^l nucleolar organizer.

A choice between the two explanations of nucleolar segregation described above is impossible without direct evidence, but the second alternative explains the presence of NLB and segregated nucleoli without invoking any hypothetical substance required for the formation of a normal nucleolus.

Our approach to the study of nucleolar structure and function involves the use of mutants carrying specific genetic lesions that result in a reduction of rRNA synthesis. Further studies with various temperature-sensitive mutations (34, 37) known to affect the production of ribosomal subunits at specific steps should provide new insights into the relationship between nucleolar structure and function. One important advantage of using nucleolar mutants for these studies is that the defect is stable, and this allows us to manipulate the cells in various ways. We have recently shown that the cells of the lethal nucleolar mutants survive in tissue culture, which opens the way for detailed morphological and biochemical studies of segregated nucleoli in vitro (20). We are also in the process of transferring nuclei of p^{l-1}/o cells to normal cells to see if the morphology of the segregated nucleolus is changed in the presence of a normal nucleus.

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