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RNA-Dependent DNA Polymerase: Possible Role in the Amplification of Ribosomal DNA in *Xenopus* oocytes

(autoradiography/2′,5′-dimethyl-*N*(4′)benzyl-*N*(4′)[desmethyl]rifampicin)

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ABSTRACT **2′,5′ - dimethyl - *N*(4′)benzyl - *N*(4′) - [desmethyl]rifampicin, a derivative of rifampicin, is used to acquire autoradiographic evidence that RNA-dependent DNA synthesis is involved in gene amplification during the early oogenesis of *Xenopus laevis*.**

Crippa and Tocchini-Valentini (1) have shown that an RNA-dependent DNA polymerase might be involved in amplification of the genes (ribosomal DNA, rDNA) that specify the sequence of ribosomal RNA during the early oogenesis of *Xenopus laevis*. Their main arguments in support of this postulate are that a large fraction of the RNA transcribed in the small oocytes occurs in a complex with rDNA, and that a rifampicin derivative (Me_2BzRif) that strongly inhibits RNA-primed DNA synthesis (2, 3) acts preferentially on the heavy DNA peak in a CsCl gradient; the heavy DNA corresponds to rDNA.

In order to identify the cells responsible for these biochemical findings and to localize more precisely the intracellular site of action of the rifampicin derivative, we have repeated, and analyzed by autoradiography, some of the biochemical experiments of Crippa and Tocchini-Valentini; such parallel control experiments were particularly important since the biochemical work has been done on whole ovaries, which contain many cells besides the oocytes. Moreover, since treatment of the isolated ovaries with the drug was for a rather long time (48 hr) in the experiments of Crippa and Tocchini-Valentini, the results could have been due to cytolysis of part of the cell population. Cytological studies were needed to check this possibility.

Several papers have shown that rDNA amplification takes place in the extrachromosomal "cap" present in the young oocytes of *Xenopus laevis* at the pachytene stage (4–7). If the conclusions of Crippa and Tocchini-Valentini are correct, Me_2BzRif ought to specifically inhibit DNA synthesis in the "caps."

Ovaries of *Xenopus laevis* tadpoles, at the time of metamorphosis, were dissected and incubated in 5 ml of Difco Medium 199 (8°C). Batches of 20 gonads were supplemented with [^{3}H]thymidine (100 μCi/ml) (The Radiochemical Center, Amersham, Bucks, England) in the presence or absence of Me_2BzRif (100 μg/ml). The drug had previously been dissolved in dimethylsulfoxide. Samples were collected after 24, 48, and 60 hr. Control determinations with Me_2SO and [^{3}H]thymidine, at the concentration used in the experiments but without the drug, were made in order to check possible toxic effects of the solvent. Fixation, sectioning, and autoradiographic procedures were described (8).

The results are illustrated in Figs. 1, 2, and 3 and quantitated in Table 1. The morphological aspect of the treated ovaries is identical with that of the control gonads during the first 60 hr of incubation. Me_2SO, at the concentration used, has no effect upon the morphology of the cells, nor on the rate of [^{3}H]thymidine incorporation. Fig. 1 represents pachytene cells that have been cultivated for 24 hr in the presence of Me_2SO, Me_2BzRif, and thymidine; after Unna staining, the "caps" stain purple red and the chromosomes green, as in the control gonads that have been fixed immediately after dissection (8). Fig. 2 is an autoradiograph demonstrating the incorporation of [^{3}H]thymidine in the ovaries after 24 hr of incubation; the nuclei of some of the follicle and other somatic cells are labeled with [^{3}H]thymidine, as are the "caps" of the pachytene cells. All the radioactivity is DNase sensitive. Fig. 3 shows a similar picture, but after treatment with Me_2BzRif; the somatic cell nuclei are normally labeled, but most of the "caps" are now unlabeled. Table 1 reports the results obtained by grain counting over the pachytene "caps,"

TABLE 1. *% of labeled pachytene "caps" taken at random in sections of Xenopus ovaries*

	Unlabeled (%)	Labeled by 2–12 grains (%)	Labeled by more than 12 grains (%)
Controls 24 hr	2	75	23
Treated 24 hr	28	63	9
Controls 48 hr	1	54	45
Treated 48 hr	66	29	5
Controls 60 hr	1	37	62
Treated 60 hr	82	18	0

Incorporation of [^{3}H]thymidine (100 μCi/ml) ± Me_2BzRif (100 μg/ml). Measurements were made on 200 nuclei of each class.

Abbreviations: rDNA, DNA coding for ribosomal-RNA; Me_2BzRif, 2′,5′-dimethyl-*N*(4)′-benzyl-*N*(4′)-[desmethyl]rifampicin; Me_2SO, dimethylsulfoxide.

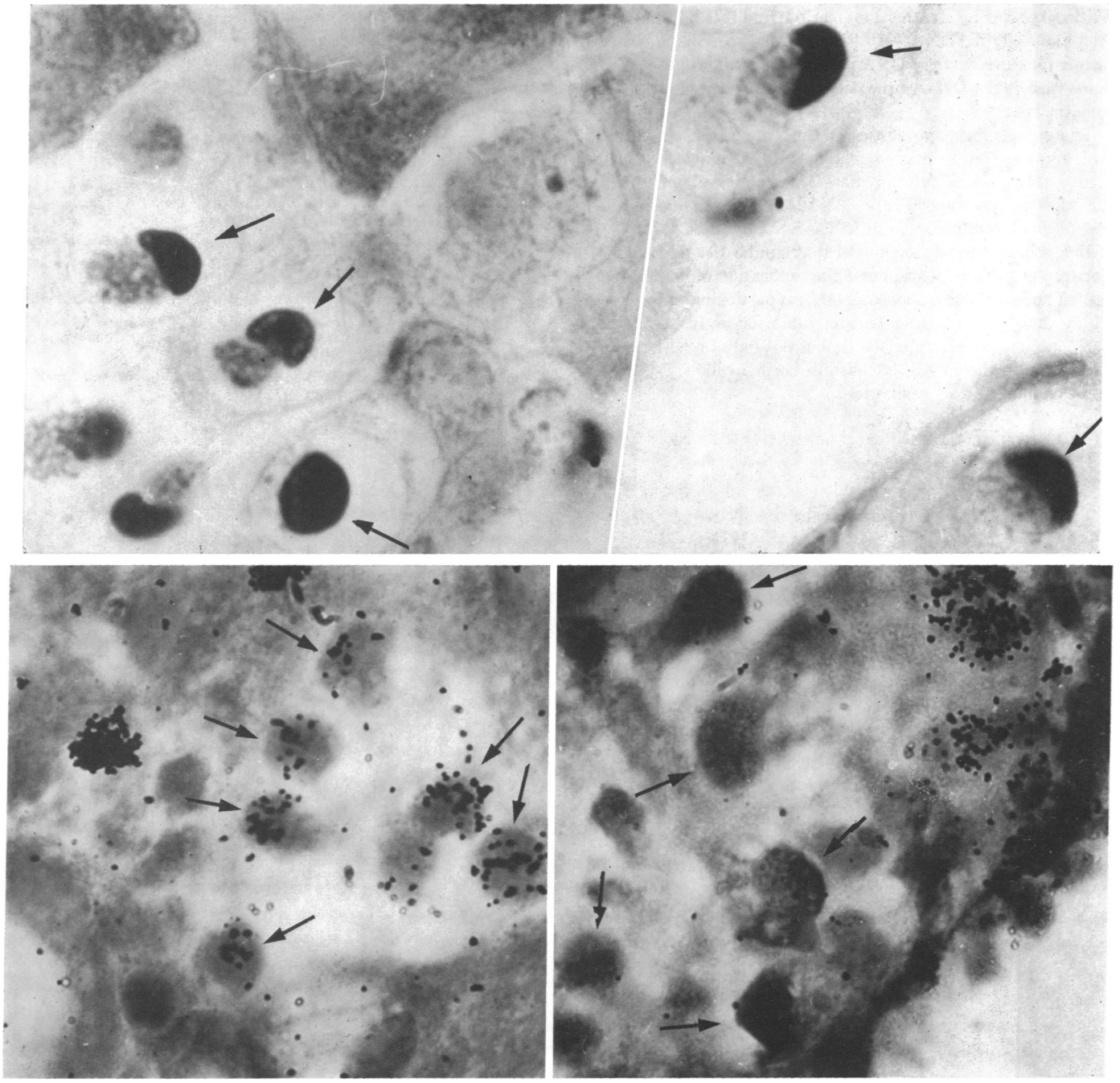

FIG. 1. (*top*) Section through an ovary of newly metamorphosed *Xenopus laevis* pachytene cells after Unna staining; the "caps" stain red (*arrows*), the chromosomes stain green.

FIG. 2. (*lower left*) Autoradiograph after [^{3}H]thymidine incubation of the gonad (100 μCi/ml for 24 hr). The nuclei of the somatic cells and the "caps" (*arrows*) are labeled.

FIG. 3. (*lower right*) Autoradiograph after [^{3}H]thymidine incubation of the gonad (100 μCi/ml for 24 hr) in the presence of Me_2BzRif (100 μg/ml). The somatic cell nuclei are normally labeled; the "caps" (*arrows*) are unlabeled.

after different periods of incubation in [^{3}H]thymidine, in the presence or absence of Me_2BzRif.

The observations made on control oocytes confirm earlier results obtained, by autoradiography, on [^{3}H]thymidine incorporation into *Xenopus* young ovaries (4–6, 8). Our results with Me_2BzRif are in good agreement with the biochemical data (1); they show that a preferential inhibition of DNA synthesis occurs in the "cap" of pachytene cells in the presence of this drug. This inhibition, which is restricted to the amplified DNA, increases with time and does not affect somatic DNA synthesis. The difference between the control and treated oocytes is particularly striking in long-term (48–60 hr) experiments, suggesting that Me_2BzRif inhibits rDNA synthesis in a progressive manner.

Ovarian rDNA of *Xenopus* behaves in many peculiar ways: it does not stain in a classical manner with Unna (8), it is

synthesized during a rather long period; and it is not methylated like somatic rDNA (9); it is, in tissue sections, a better primer for the Kornberg DNA polymerase (A.F., unpublished data) than is the DNA of the oocyte chromosomes and of the somatic cells.

The cytochemical properties of the "cap" DNA (staining with pyronine, even after digestion with ribonuclease, good primer activity for DNA polymerase) are compatible with the idea that a large proportion of rDNA is in the form of replicating single-strand molecules or DNA–RNA hybrids.

It has been recently suggested that similarities might exist between gene amplification and the replication of an episome (10). The fact that synthesis of rDNA is particularly sensitive, at the amplification stage, to an inhibitor of RNA-primed DNA polymerase [an enzyme that is not only found in RNA viruses and cancer cells, but also in normal cells (11)], lends some support to this suggestion.

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