percent pyruvic acid) in the latter polysaccharide was known (*4*, *8*).

These results show that pyruvate groups, which have also been found in agar (1 percent) (*9*) and in polysaccharides of *Corynebacterium insidiosum* (*10*), *Klebsiella rhinoscleromatis* (8.3 percent (*11*), a *Pseudomonas* strain (4.3 percent) (*8*), and in the polysaccharides of all *Xanthomonas* species so far examined (1.0 to 7.6 percent) (*8*), are also important substituents in the polysaccharides of *Rhizobium trifolii* and *R. meliloti* and function as a major determinant in their serological specificity. Pyruvate groups or pyruvylated sugars also appear to account for the cross-reactivity between antiserum to pneumococcal type 27 and the diverse polysaccharides containing pyruvate substituents; the type 27 polysaccharide had been shown to contain glucose, galactose, rhamnose, glucosamine, and phosphate (*12*). However, despite the presence of pyruvate and acetyl groups and the similarity of their sugar composition, it is still surprising that the polysaccharides of *R. trifolii* show very little cross-reactivity with each other.

In that current views ascribe a large part to polysaccharides in the specificity of the interaction between *Rhizobium* species and their plant hosts (*13*), our findings raise questions as to whether the newly demonstrated acyl groups or the corresponding acylated sugars are mediators of these, as well as of immunological specificities.

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Visualization of Nucleolar Genes

Abstract. *The presence of extrachromosomal nucleoli in amphibian oocytes has permitted isolation and electron microscopic observation of the genes coding for ribosomal RNA precursor molecules. Visualization of these genes is possible because many precursor molecules are simultaneously synthesized on each gene. Individual genes are separated by stretches of DNA that apparently are not transcribed at the time of synthesis of precursor rRNA in the extrachromosomal nucleoli.*

During early growth of the amphibian oocyte, the chromosomal nucleolus organizer is multiplied to produce about a thousand extrachromosomal nucleoli within each nucleus (*1*). There is convincing evidence that these nucleoli function similarly to chromosomal nucleoli in the synthesis of rRNA precursor molecules (*2*). In thin sections of fixed oocytes, each extrachromosomal nucleolus typically shows a compact fibrous core surrounded by a granular cortex (Fig. 1). Previous studies have shown that only the core region contains DNA, whereas both components contain RNA and protein (*3*). The large size of the amphibian oocyte nucleus (*4*) allows rapid isolation and manipulation of the extrachromosomal nucleoli before extensive denaturation and cross-linking of proteins occurs. If saline of low molarity or deionized water is used as the isolation medium, nucleolar cores and cortices can be separated and the DNA-containing cores dispersed for electron microscopy (*5*).

Each unwound isolated nucleolar core consists of a thin axial fiber, 100 to 300 Å in diameter, that is periodically coated along its length with matrix material (Figs. 2 and 3). The axial fiber of each core forms a circle, and treatment with deoxyribonuclease breaks the core axes. The diameter of trypsin-treated axial fibers (about 30 Å) suggests that the core axis is a single double-helix DNA molecule coated with protein (*6*). The matrix segments along a core axis exhibit thin to thick gradations, and show similar polarity along the axial fiber. Each unit is separated from its neighbors by matrix-free axis segments.

Nucleolar core axes are stretched to variable degrees depending on preparative procedures. For example, drying preparations out of deionized water before staining causes little or no stretching of axial cores (Fig. 2), whereas precipitating preparations with acetone staining solution before drying stretches the core axes to varible degrees over the grid surface (Fig. 3). When regions of core axes appear unstretched or uniformly stretched, the matrix units along a specific region are similar in length; unstretched matrix units are 2 to 2.5 μ long but can be 5 μ long after severe stretching. The matrix-free segments between matrix units also show variations in length due to stretching, but, in addition, exhibit differences in length independent of stretching (Fig. 3). Most matrix-free segments are about one-third the length of adjacent matrix units, but bare regions up to ten times as long as neighboring matrix

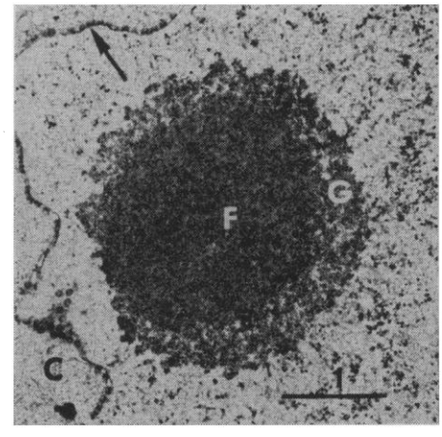

Fig. 1. Thin section of extrachromosomal nucleolus from *Triturus viridescens* oocyte. A granular cortex (*G*) surrounds a compact fibrous core (*F*). Portions of the nuclear envelope (arrow) and cytoplasm (*C*) are visible. Conventional osmium tetroxide fixation, Epon embedding, and uranyl acetate staining. Scale, 1 μ.

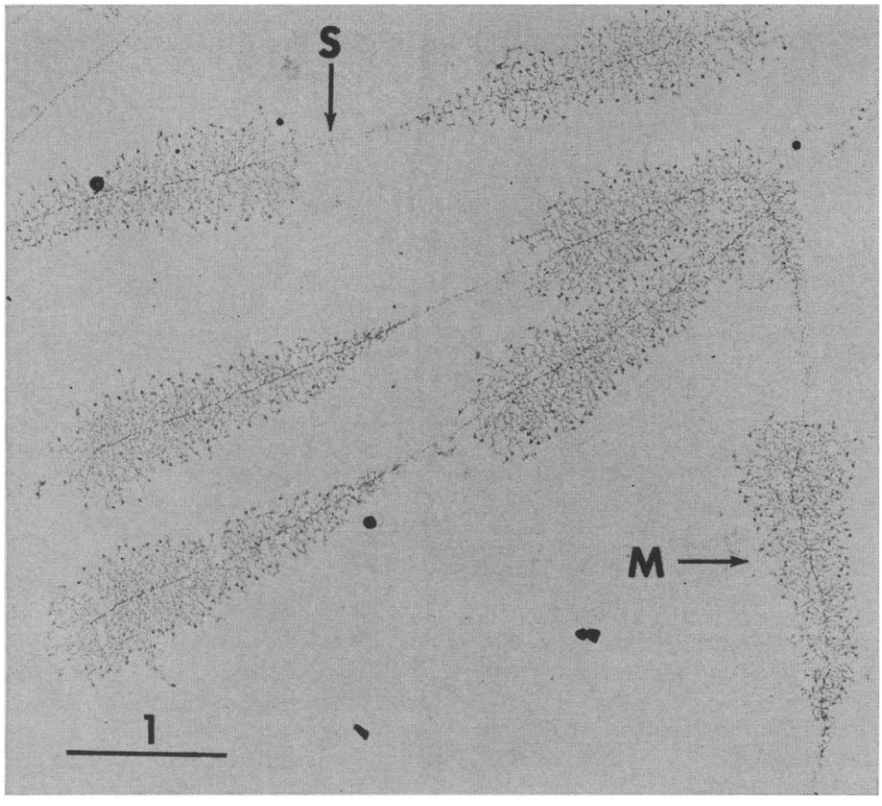

Fig. 2. Portion of a nucleolar core isolated from *Triturus viridescens* oocyte showing matrix units (*M*) separated by matrix-free segments (*S*) of the core axis. The axial fiber can be broken by treatment with deoxyribonuclease, whereas the matrix fibrils can be removed by ribonuclease, trypsin, or pepsin. The specimen was prepared by placing the contents of an oocyte nucleus in deionized water, thus causing dispersal of nucleolar components; the unwound cores were centrifuged through a neutral solution of 0.1*M* sucrose with 10 percent formalin onto a carbon-covered grid; the grid was rinsed in 0.4 percent Kodak Photo-Flo before drying; the preparation was then stained for 1 minute with 1 percent phosphotungstic acid in 50 percent ethanol at *p*H 2.5 (unadjusted) rinsed in 95 percent and then in 100 percent ethanol, and dried with isopentane. The matrix units and intermatrix segments apparently are unstretched by this procedure. Scale, 1 μ.

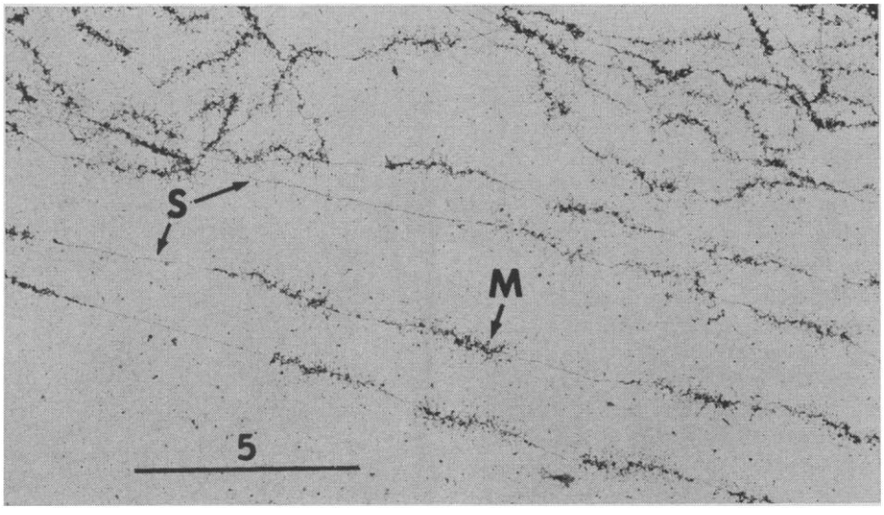

Fig. 3. Portion of nucleolar core isolated from *Triturus viridescens* oocyte, showing matrix units (*M*) separated by matrix-free segments (*S*) of the core axis. Intermatrix segments of various lengths are present. Specimen preparation was similar to that described in Fig. 2, except that the centrifuged specimen was rinsed in water and dipped without drying into 1 percent uranyl acetate in 80 percent acetone and stained for 5 minutes. With this procedure, the matrix units and matrix-free segments appear stretched—slightly to severely—depending on their location on the grid and on how tightly the centrifugation step has compressed them to the grid surface. Scale, 5 μ.

units have been observed in both *Xenopus laevis* and *Triturus viridescens*. There appears to be no pattern to the distribution of the longer matrix-free regions along the core axis.

Detailed examination of matrix units shows that each consists of about 100 thin fibrils connected by one end to the core axis and increasing in length from the thin to the thick end of the unit (Fig. 2). Treatment with ribonuclease, trypsin, or pepsin removes the matrix fibrils from the core axis. After labeling of RNA in intact oocytes for 30 to 60 minutes with tritiated ribonucleosides, electron microscopic autoradiography of unwound cores shows silver grains only over the matrix units. This initial incorporation corresponds in time to the appearance of labeled 40*S* precursor rRNA molecules in nuclear fractions from amphibian oocytes (*7*). Furthermore, the length of unstretched to slightly stretched units (2 to 3 μ) is in close agreement with the length of DNA required to code for the precursor molecule synthesized in amphibians (*8*). Therefore, we believe that each matrix-covered DNA segment is a gene coding for rRNA precursor molecules.

The mechanism of RNA polymerase action in DNA-dependent synthesis of RNA involves a polymerization of monomer ribonucleotides into a polyribonucleotide chain that is immediately dissociated from the template DNA strand (*9*). The structural arrangement of the fibrils in each matrix unit is consistent with a model in which numerous RNA molecules are sequentially initiated before completion of the first. Thus, visualization of the genes coding for rRNA precursors is possible because many molecules are simultaneously synthesized on each gene. In that either ribonuclease or proteases remove matrix fibrils from the core axis, each fibril probably consists of a growing rRNA precursor molecule coated with protein.

If each ribonucleoprotein matrix fibril contains one growing rRNA precursor molecule, the number of fibrils per matrix unit (about 100) and the dimension of the RNA polymerase molecule in the axis of transcription (about 100 Å) (*10*) indicate that about one-third the length of each gene coding for rRNA precursors is covered with polymerase molecules (*8*). A high concentration of RNA polymerase on rRNA genes also has been reported for *Escherichia coli* (*11*).

In equilibrium gradients, the nu-

cleolar DNA of *X. laevis* can be separated from most of the DNA of the cell as a heavier peak (G•C satellite) (*12–14*). Saturation hybridization (*12, 14*) indicates that about 40 percent of the G•C satellite codes for rRNA (*15*). Annealing experiments with fractionated satellite DNA show that the stretches of DNA coding for 18*S* and 28*S* rRNA are alternating, closely adjoining, but separated by stretches of DNA higher in G•C content and not homologous to rRNA (*13, 14*). The latter observations agree with the evidence that each rRNA precursor molecule consists of one 18*S* and one 28*S* rRNA molecule, plus a portion that is degraded during the formation of the two rRNA molecules (*13, 16*).

We propose that the redundant structural arrangement of the rRNA precursor genes and intergene segments seen in isolated nucleolar cores visually confirms the biochemical nature of nucleolus organizer DNA in amphibians. Thus, the DNA axis of the matrix-covered segments corresponds to the satellite portion that is homologous to the entire precursor rRNA molecule (that is, homologous to one 18*S* and one 28*S* rRNA molecule plus the degraded part of the precursor rRNA molecule), and the DNA in the intergene regions corresponds to the remaining portion of the satellite. Measurements of relative lengths of matrix-free and adjacent matrix-covered units in *X. laevis* show that the mean length of intergene segments is about two-thirds the length of a precursor rRNA gene. This indicates that approximately 40 percent of the G•C satellite is inactive nucleolar DNA and about 60 percent consists of genes coding for precursor molecules.

Although the structure of chromosomal loci synthesizing RNA has already been documented, we believe ours are the first observations of the structure of individual genes and associated transcription products whose specific function is known—namely, the extrachromosomal nucleolar genes on which rRNA precursor molecules are synthesized.

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5. Details of techniques are given in legends for Figs. 2 and 3. Oocytes of *Xenopus laevis,* the African clawed toad, and *Triturus viridescens,* the spotted newt of eastern North America, were used in these studies. Limited examinations in two other genera, *Rana* and *Plethodon,* indicate that these observations probably extend to all amphibians. Earlier reports of these results are found in: O. L. Miller, Jr., and B. R. Beatty, *J. Cell Biol.* **39**, 156a (1968); ———, in *Handbook of Molecular Cytology,* A. Lima-de-Faria, Ed. (North-Holland, Amsterdam, in press); ———, *Genetics,* in press.
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Anabolic Steroid: Effects on Strength Development

Abstract. *Twelve matched pairs of subjects, fed a high protein diet, were trained with weights for 6 weeks. In the final 3 weeks twelve subjects received 5 milligrams of methandrostenolone (Dianabol) twice daily. Maximum weight lifting, thickness of skin folds, oxygen uptake, blood chemistry profile, and concentration of blood lipids were determined. Also used were cable tensiometry and anthropometric measurements. The strength of treated subjects increased significantly; their mean weight gain was 2.48 kilograms with no significant change in skin fold thickness. Several anthropometric measurements increased significantly, as did oxygen uptake ability and nitrogen retention by the blood.*

Use of anabolic steroids by athletes attempting to develop strength has become increasingly widespread, especially by those in activities where strength is the prime factor for successful performance. Although many instances of extraordinary and rapid improvement have been reported, the evidence appears entirely empirical. Little is known about possible long-term side effects on adults. However, physically immature individuals can expect irrevocable and irreversible developments. Possible acceleration of epiphyseal ossification (*1*) and manifestations resembling macrogenitosomia precox (*2*) are two of the severe contraindications in the use of steroids by teen-agers.

Anabolic agents have been used for some time for patients recovering from illness or after surgery, for treatment of osteoporosis, fracture healing, severe burns, and muscular dystrophy, for protein tissue building, and for myotrophism. Anabolic agents have also been credited with having an important effect in stimulating the appetite and imparting a feeling of well-being (*3*). Although there is little data on the use of anabolic steroids by athletes, there is sufficient clinical evidence that the anabolic potencies of these drugs should, on theoretical grounds, stimulate muscle hypertrophy and strength increases in normal healthy men. Androgens do exert a positive effect on muscle growth in various animal species; for example, hypertrophy of certain muscles in immature guinea pigs accompanied administration of testosterone propionate (*4*), and weekly intramuscular injection of 1 mg of testosterone per kilogram of body weight resulted in increased weight gains in steers (*5*).

It was difficult to find volunteers who were willing to take the steroid. Many considered participation but were apprehensive because of the paucity of knowledge concerning side effects. There appears to be a widespread rumor that steroid treatment reduces the sexual drive. Thus we changed our