These studies show that linear pSLA2 plasmids are replicated primarily from a site near the center of the plasmid and not by full-length strand displacement initiated at the telomeres. The ends of replicating pSLA2 DNA molecules contain 3′ overhangs, and thus differ from the blunt-ended telomeres found on previously identified linear replicons that use 5′ DNA binding proteins to prime DNA synthesis. We suggest that synthesis of the 5′ terminal DNA segment of the lagging strand of pSLA2 DNA is primed, on a template that consists of the 3′ overhang of the leading strand, by the protein found earlier to be covalently attached to the 5′ termini of mature pSLA2 DNA (*15*). However, DNA synthesis initiated at the telomeres of pSLA2 does not proceed continuously through the entire molecule, unlike the protein-primed DNA synthesis that occurs in adenovirus and bacteriophage ϕ29. Instead, only a 280-nucleotide single-strand segment is filled in. Whereas internally initiated DNA replication has also been observed in certain bacteriophage linear replicons (such as T7), completion of the terminal portion of the lagging strand is accomplished by redundancy of the telomeric sequence (*16*) rather than by protein-primed DNA synthesis.

The chromosomes of several *Streptomyces* species are linear (*17*). As the inverted repeat sequences at the telomeres of the *S. lividans* chromosome are highly similar to those of pSLA2, pSCL, and other *Streptomyces* linear plasmids, we speculate that the replication mechanism found for pSLA2 may be used more generally for DNA synthesis in *Streptomyces*.

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22. We thank H. Hirochika and K. Sakaguchi for *S. rochei* strain 7434-AN4. Supported by grants from the NIH and the American Cancer Society (S.N.C.) and by a postdoctoral fellowship from the Ministry of Education of the Republic of China (P.-C.C.).

3 February 1994; accepted 28 June 1994

Ribosomal Heterogeneity from Chromatin Diminution in *Ascaris lumbricoides*

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The genome of *Ascaris lumbricoides* encodes both germline- and soma-specific proteins homologous to the eukaryotic small ribosomal protein (Rp) S19. The two *Ascaris* homologs differ by 24 amino acid substitutions and are both components of the small ribosomal subunits. In oocytes, the germline RpS19 homolog (RpS19G) predominates. During chromatin diminution, however, the gene is eliminated from all presomatic cells, and RpS19G is replaced by the product of the somatic gene (RpS19S). Chromatin diminution in *A. lumbricoides* causes a change in the protein composition of ribosomes during development and represents an alternative means of gene regulation.

Chromatin diminution in nematodes, which discard genomic DNA from all presomatic cells during early embryonic development, represents an exception to the DNA "constancy" rule. This process has been reported in about a dozen Ascaridae species (*1*, *2*), which are nematodes that parasitize vertebrates and some invertebrates (*3*). In *Ascaris lumbricoides*, the eliminated material contains large amounts of highly repetitive satellite DNA and also certain amounts of less repetitive and single-copy DNA sequences (*1*, *4*). One expelled gene (*alep-1*) encodes a putative protein (RpS19G) homologous to the small ribosomal subunit protein S19 of eukaryotes (*5*). The *alep-1* gene is transcribed in all cells of the germ line, but no *rpS19G* transcripts are found in somatic cells, from which the gene is absent (*5*).

The 148–amino acid *rpS19G* gene product is a component of the small subunit of the *Ascaris* oocyte ribosomes (Fig. 1A), as we predicted from its sequence. Although the protein was absent from the ribosomes of muscle cells, antiserum to RpS19G (*6*) detected another protein that had a slightly reduced mobility on gels. A weak band of similar mobility was also extracted from oocyte ribosomes (Fig. 1A). The almost identical molecular size and similar antigenic properties suggested that the larger protein is an RpS19 isoform, which may be encoded by a gene that is not eliminated by chromatin diminution and is present in both the soma and germ line.

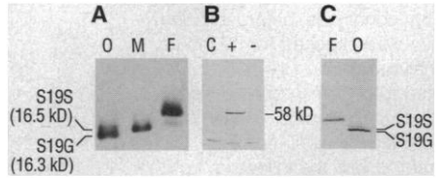

Fig. 1. Distribution of *A. lumbricoides* RpS19G and RpS19S. (**A**) Protein immunoblot with ~4 μg per lane of sucrose-purified small ribosomal subunits from *A. lumbricoides* oocytes (O) and muscles (M) and an aliquot of a bacterial protein extract containing the RpS19G fusion protein (F). The concentration of the small ribosomal subunits was standardized on standard two-dimensional gels (*17*). (**B**) Protein immunoblot of the RpS19S fusion protein. RpS19G antibodies detected the 58-kD RpS19S fusion protein (*18*) in IPTG-induced TB1 cells containing the fusion vector (+), but not in noninduced cells (−) or in induced cells containing the empty expression vector pMalC2 (C). (**C**) Total protein extract from *Ascaris* oocytes (O) and an aliquot of the RpS19G fusion protein as control (F). Isolation and purification of small ribosomal subunits from *A. lumbricoides* oviducts or muscle tissues were performed as described (*19*). Protein extracts were analyzed by electrophoresis with a 16%T (total concentration of monmers), 6%C (cross-linking agent) polyacrylamide gel. Protein transfer and immunoblotting with the serum to a bacterially expressed RpS19G fusion protein (*6*) were done as described (*20*). A secondary antibody, an alkaline phosphatase–conjugated goat antibody to rabbit immunoglobulin (Dako), was used.

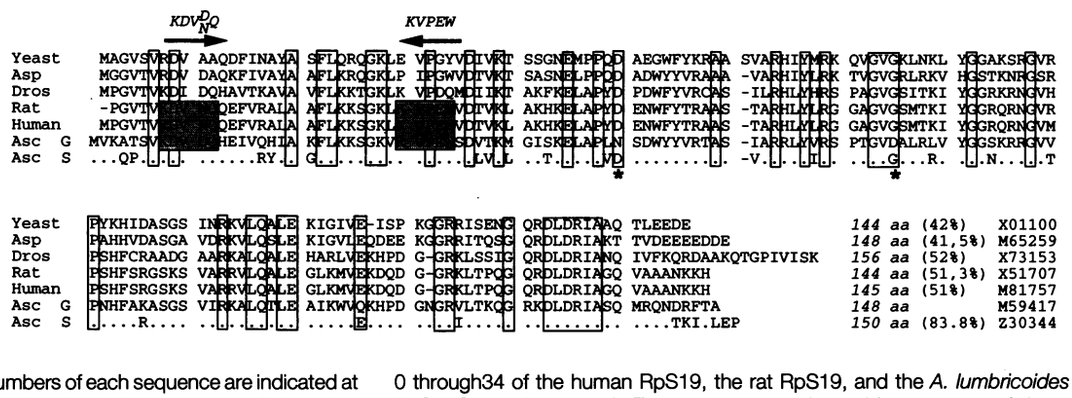

Fig. 2. Alignment of eukaryotic RpS19 homologs. The amino acid sequences from *Saccharomyces cerevisiae* RpS16 (yeast) (*21*), *Aspergillus nidulans* RpS16 (Asp) (*22*), *Drosophila melanogaster* RpS19 (Dros) (*23*), rat RpS19 (*24*), human RpS19 (*25*), and the *A. lumbricoides* RpS19G (*5*) and RpS19S are compared. The lengths, the sequence similarities with RpS19G in percent of identical amino acids (aa), and the GenBank–European Molecular Biology Laboratory accession numbers of each sequence are indicated at the end of the sequences. Conserved amino acids are boxed. Dots in the sequence of RpS19S indicate those amino acids identical with those in RpS19G. Asterisks denote amino acid changes in RpS19G at positions conserved in all other eukaryotic RpS19 sequences. Degenerate primers for the PCR amplification were derived from amino acid positions 8 through 12 and 0 through34 of the human RpS19, the rat RpS19, and the *A. lumbricoides* RpS19G (shaded boxes). The consensus amino acid sequences of these regions are indicated on top of the first line. Arrows show the orientation of the deduced degenerate primers (*7*). Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

To identify the gene, we carried out the polymerase chain reaction (PCR) on total muscle RNA (*7*). We subcloned a PCR product with the expected size of 81 base pairs (bp) and used it to identify a corresponding full-length 650-bp complementary DNA (cDNA) clone (*rpS19S*) from an *Ascaris* cDNA library. The cDNA includes a 5′ spliced leader from *Ascaris* (*8*), a 3′-polyadenylate sequence, and a single open reading frame that encodes a 150–amino acid protein homologous to other RpS19 sequences (Fig. 2). RpS19S shares 124 identical amino acids with the *Ascaris* RpS19G isoform (83.8% identity). Twenty-four amino acids, dispersed throughout the protein, are different; 12 of the differences are conservative changes. Of the 24 replacements, 21 are in regions not conserved in the eukaryotic RpS19 family, and the remainder involve amino acid changes in RpS19G at positions strictly conserved in the other eukaryotic RpS19 sequences (Fig. 2). Both *Ascaris* RpS19 isoforms, as well as the other eukaryotic RpS19 homologs, contain multiple basic amino acids and have a deduced isoelectric point above 11.

Three lines of evidence suggested that RpS19S corresponds to the fainter band of slower mobility detected by protein immunoblotting (Fig. 1A). First, a bacterial RpS19S fusion protein was specifically recognized by antibodies to RpS19G (Fig. 1B). Second, we predicted from the gel mobilities that the protein would be two amino acids longer than RpS19G. Finally, RpS19S was encoded by a single copy gene that was not eliminated during chromatin diminution (Fig. 3) and that was expressed in all developmental stages tested (Fig. 4A).

Ascaris somatic ribosomes included only RpS19S (Fig. 1A), whereas both RpS19 homologs were incorporated into the small ribosomal subunits of the germ line. Ribosomes containing RpS19G, however, were about 10 times more abundant than those with RpS19S (Fig. 1A). In oocytes, the RpS19 isoforms occurred at a molecular ratio that corresponds roughly to the distribution of the two ribosomal classes (Fig. 1, A and C). Thus, ribosomal assembly may depend on the relative concentration of the two RpS19 isoforms in the cell, rather than on preferential incorporation of RpS19G.

However, in the germ line both *rpS19* genes were equally transcribed, and the amount of *rpS19S* mRNA did not change significantly between the germ cells and somatic tissues, relative to *rpL7* mRNA (encoding an Rp of the large subunit) we used as a control (Fig. 4B). Therefore, the shift of RpS19S production takes place at the translational level. In germ cells, the *rpS19G* mRNA appears to be preferentially selected for translation. After elimination of the *rpS19G* gene, the mRNA disappeared (Fig. 4B) and the *rpS19S* mRNA became solely available for translation. RpS19S production is therefore accomplished by a combination of translational control and chromatin diminution—an alternative means to gene regulation at the transcriptional level.

The genome of *Parascaris univalens*, an intestinal parasite of the horse, also encodes two RpS19 isoforms, one of which is restricted to the germ line (Fig. 3) (*5*). Thus not only the sequence similarities of the two *rpS19* genes, but also their behavior during the elimination process, have been maintained during the evolution of the two nematode species. The two *Ascaris* RpS19 isoforms shared less sequence homology with each other than they did with their homologs from *Parascaris* (Fig. 3) (*5*). We therefore suggest that the two *rpS19* genes resulted from a gene duplication that occurred before the two nematode species were separated in evolution.

Nothing is known about the specific function of the eukaryotic RpS19 protein family. In rat liver ribosomes, RpS19 has been localized on the external surface of the 40*S* subunit in the bill of the head and near

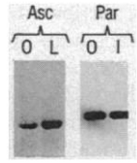

Fig. 3. Genomic organization of the *rpS19S* gene. Southern (DNA) blot analysis with Eco RI–digested total *A. lumbricoides* (Asc) and *Parascaris equorum* (Par) DNA from oocytes (O), larvae (L), or intestines (I) hybridized with an *rpS19S* cDNA probe. Genomic *Ascaris* and *Parascaris* DNAs were isolated as described (*5*). The probes were nick-translated, and Southern blots were hybridized by standard techniques (*16*). The *Ascaris* DNA filter was washed three times for 20 min each in 0.1× standard saline citrate (SSC) and 0.1% SDS at 60°C (high stringency). The *Parascaris* DNA filter was washed at 42°C, first for 20 min with 0.5× SCP (20× SCP is 2 M NaCl, 0.6 M Na_2HPO_4, and 0.02 M EDTA, pH 6.2) and 0.5% SDS and then with two washes with 2× SSC and 0.1% SDS (moderate stringency).

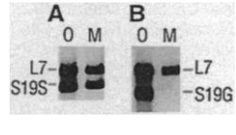

Fig. 4. Distribution of *rpS19G* and *rpS19S* mRNA. Total RNA from oocytes (O) and muscle tissue (M) was hybridized simultaneously with *rpS19S* and *rpL7* cDNA probes (**A**) or rpS19G and rpL7 cDNA probes (**B**). Total RNA from *Ascaris* oocytes and muscle tissue was isolated as described (*5, 26*). Denatured RNA samples (15 μg) were analyzed by gel electrophoresis in a denaturing 1% agarose gel (*26*), transferred to a membrane (Genescreen Plus, DuPont), cross-linked with an ultraviolet cross-linker (Bio-Rad), and hybridized as described (*16*).

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the binding site of initiation factor eIF-2 (*9*). The sequence of RpS19 is highly conserved among nematodes, insects, vertebrates, yeast (Fig. 2), and archaebacteria (*10*), and the homologous yeast protein Rp16A is essential for viability (*11*).

Developmentally controlled ribosomal heterogeneities attributed to changes in ribosomal proteins have been identified in the cellular slime mold genera *Dictyostelium* and *Polysphondylium* (*12*). In these organisms, ribosomal switches occur at discrete stages of the life cycle. Ribosome heterogeneity was also discovered in human males, where two genes coding for RpS4 isoforms exist on the sex chromosomes, one on the Y and one on the X, suggesting that the ribosomes of human males and females differ (*13*). At different stages during the life cycle of *Plasmodium berghei* and *P. falciparum*, two structurally distinct 18S ribosomal RNA genes are expressed (*14*). In *Xenopus laevis*, two types of 5S ribosomal RNA genes exist, both of which are expressed in oocytes, but only one type of which is expressed in somatic cells (*15*).

At present, it is quite unclear why certain eukaryotic organisms make distinct populations of ribosomes at discrete stages of their life cycle. Because chromatin diminution in *A. lumbricoides* parallels germline-soma differentiation, the change in ribosomes may affect somatic cell differentiation, perhaps by controlling translation of somatic genes. Alternatively, the ribosomal heterogeneity may reflect different quantitative requirements of protein synthesis of the two cell lineages; an adult *Ascaris* female produces as many as 200,000 eggs per day (*3*), whereas somatic cells do not require such a high rate of protein synthesis.

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27. We thank F. Amaldi, T. Nilsen, J. Stahl, and M. Wymann for discussions; all the colleagues in our laboratory for comments on the manuscript; and J. Oberson, L. Codourey, H. Gachoud, Y. Molleyres, and A. Tempini for technical assistance. Supported by the Swiss National Science Foundation by grants 3.141-0.88 and 31.31001.91, by the SANDOZ Stiftung zur Förderung der Medizinisch-Biologischen Wissenschaften, and by the ROCHE Research Foundation.

10 March 1994; accepted 17 June 1994

Inhibition of NF-κB by Sodium Salicylate and Aspirin

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The transcription factor nuclear factor–κB (NF-κB) is critical for the inducible expression of multiple cellular and viral genes involved in inflammation and infection including interleukin-1 (IL-1), IL-6, and adhesion molecules. The anti-inflammatory drugs sodium salicylate and aspirin inhibited the activation of NF-κB, which further explains the mechanism of action of these drugs. This inhibition prevented the degradation of the NF-κB inhibitor, IκB, and therefore NF-κB was retained in the cytosol. Sodium salicylate and aspirin also inhibited NF-κB–dependent transcription from the Igκ enhancer and the human immunodeficiency virus (HIV) long terminal repeat (LTR) in transfected T cells.

The salicylates, or aspirin-like drugs, are widely prescribed agents used to treat inflammation (*1*). Their effectiveness has been attributed to their ability to inhibit prostaglandin production by inhibiting the cyclooxygenase, prostaglandin H (PGH) synthase (*2*). However, doses of aspirin necessary to treat chronic inflammatory diseases are much higher than those required to inhibit prostaglandin synthesis (*1, 3*). Furthermore, whereas aspirin inhibits PGH synthase by acetylating it, salicylic acid, which lacks an acetyl group and is ineffective as a PGH synthase inhibitor, is nevertheless able to reduce inflammation at comparable doses to aspirin (*1, 3*). Salicylic acid also plays a role in transcription (*4, 5*). In plants, salicylic acid influences transcription of the pathogenesis-related (PR) genes in response to infection and injury (*4*), and in HeLa cells sodium salicylate activates the human heat shock transcription factor (HSTF) (*5*).

NF-κB is an inducible eukaryotic transcription factor of the *rel* family (*6*). It exists in an inactive form in the cytoplasm of most cells where it is bound to an inhibitory protein, IκB. NF-κB is activated in response to a number of stimulants including bacterial lipopolysaccharide (LPS), double-stranded RNA, phorbol esters, IL-1, and tumor necrosis factor–α (TNF-α) (*6*). Stimulation triggers the release of NF-κB from IκB, resulting in the translocation of NF-κB from the cytoplasm to the nucleus where NF-κB binds to DNA and regulates transcription of specific genes. Most of the genes known to be activated by NF-κB

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