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Reviews

Germ line – soma differentiation in *Ascaris:* A molecular approach

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Summary. The early ontogenetic segregation of germ line and somatic cells in the animal kingdom is phylogenetically very old and represents probably the first step in differentiation. While this phenomenon has been shown to occur in various animal phyla, it seems to be completely missing in the plant kingdom. In several animal species, the segregation of the germ and somatic cell lines is accompanied by the loss of whole or parts of chromosomes in the presumptive somatic cells. The cause of the chromatin diminution process as well as the significance of the germ line limited DNA sequences in species undergoing chromatin or chromosome loss still remain unknown. However, using modern biochemical and molecular biological techniques, it has become possible to analyze the process of chromatin diminution and the composition of the germ line specific DNA sequences at the molecular level.
In *Ascaris lumbricoides,* about a quarter of the total amount of germ line DNA is eliminated from the presumptive somatic cells during chromatin diminution. Hybridization experiments revealed that germ line and somatic DNA contain the same percentage of 18S+28S rRNA genes. Therefore, chromatin diminution does not serve to discard large amounts of rRNA-coding genes from the germ line cells. On the other hand, over 99%, but not all satellite DNA sequences present in the germ line genome, are expelled from the presumptive somatic cells by chromatin diminution. Molecular cloning and sequence analysis of different restriction endonuclease fragments isolated from the germ line satellite DNA indicated that this eliminated satellite is composed of a whole set of related variant sequences, which differ by small deletions, insertions and single base substitutions. Members of the same variant class are tandemly linked and therefore physically separated from other variant classes. By comparing all the determined sequences, it was possible to establish a 121 bp long and AT rich consensus sequence which itself exhibits an 11 bp long internal short range periodicity. We have no indication for transcriptional activity of the satellite DNA sequences at any stage or tissue tested. Evidence is accumulating that the eliminated DNA contains also other DNA sequences apart from the class of highly repetitive satellite DNA.
Key words. Germ line – soma differentiation; chromatin diminution; gene loss; satellite DNA; molecular embryology; nematology.

Introduction

As early as 1885, the great cell biologist August Weismann[49] formulated the germ line theory, stating that the germ line and the somatic cells segregate early and develop independently of each other. In contrast to the protozoa, where germ cells and somatic cells are generally identical and potentially immortal, the two cell lines become segregated in metazoans, leaving only the germ line cells potentially immortal. The differentiation of multicellular organisms in germ and somatic cells is phylogenetically very old and probably represents the first step in specialization. The many-celled green alga *Volvox* is composed of just two different cell types; somatic cells which are all morphologically alike and perform the vegetative functions of the organism, and the generative cells which serve for the organism's reproduction.
In the animal kingdom, early separation of germ line and somatic cells during the embryonic development is phylogenetically widespread (nematodes, arthropods, vertebrates, and others), whereas no such separation in the embryonic development of plants has been observed. The cells forming the germ line derive always from the posterior or vegetative pole of the eggs, a region that has been shown to contain in many cases specific cytoplasmic inclusions such as germinal plasm, polar granules or fibrogranular bodies (see Beams and Kessel[6], Eddy[14], Mahowald and Boswell[25] and Nieuwkoop and Sutasurya[33] for review). It has been demonstrated that this cytoplasm has the potential to induce nuclei to form primordial germ cells which later on become functional gametes[22,23].
While the segregation of the germ line and somatic cells always takes place early in ontogeny, it nevertheless occurs in different ways: The cell lineage of primordial germ cells can be traced back either to one single cell or one single nucleus (e.g. *Ascaris megalocephala, Mayetiola destructor*), or to a few cleavage cells or nuclei (e.g. *Xenopus laevis, Drosophila melanogaster,* cf. fig. 1). The future germ cells are thus not always linearly descending from a single, specific stem cell or nucleus.
In some major animal groups, the segregation of the germ line and the somatic cells is accompanied by elimination of chromosomes or diminution of chromatin in the presumptive somatic cells (cf. fig. 2). Chromatin or chromosomal loss have been shown to occur in several ciliated protozoa, nematodes, crustaceans, five different systematic categories of insects and even in one species of Acarina[32]. The phenomenon is therefore phylogenetically widespread but not universal. Boveri[8] was the first who discovered the process of chromatin diminution in the parasitic nematode *Ascaris megalocephala* (fig. 3). In the meantime, this phenomenon has been detected in 10 more nematode species (cf. table). However, not all nematodes show chromatin diminution; using classical cytological methods, Walton[48] found no indication of

chromatin diminution in at least nine parasitic nematode worms. Moreover, modern molecular and biochemical investigations also revealed no signs for the presence of chromatin loss in the free-living nematode *Caenorhabditis elegans*[16,40].

The significance of chromatin or chromosomal loss from the genome of presumptive somatic cells is still obscure. Although the somatic cells of most organisms seem to retain the full genomic content during the course of development and differentiation, there is no a priori reason why they should. In principle, only the germ line cells destined to give rise to all future eggs and sperms must contain a complete genome. Every species undergoing chromatin or chromosome loss in the presomatic cells clearly demonstrates that it carries in its germ line a chromatin fraction which is not needed for the proper function of the somatic cells. The important question of course is whether the germ line limited DNA sequences have any function in the germ line and if so, what their exact function is. The transplantation of somatic nuclei with the reduced DNA content into enucleated eggs could give us an answer. However, such experiments are technically very difficult to perform since *Ascaris* eggs are rather small and their eggshells are extremely tough.

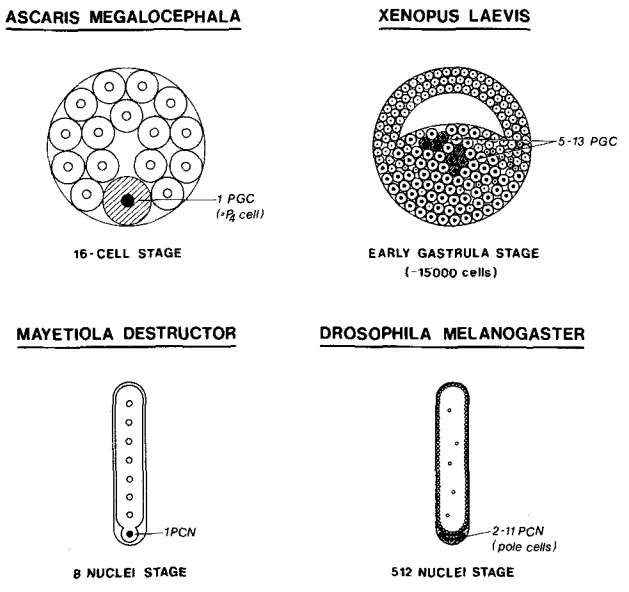

Figure 1. Segregation of germ line and somatic cells in four different animal species. The cell lineage of primordial germ cells (PGC) can be traced back to one single cell in the 16-cell stage of *Ascaris megalocephala*[9] or to one single primordial cell nucleus (PCN) in the 8 nuclei stage of the gall midge *Mayetiola destructor*[5]. In *Xenopus laevis* and in *Drosophila melanogaster*, the cell lineage of primordial germ cells cannot be traced back to one single cell or nucleus, but to 5–13 PGCs in the early gastrula stage of *Xenopus*[50] or to 2–11 pole cells in *Drosophila*[38], respectively. The number of cells in the early gastrula stage of *Xenopus laevis* is taken from Gerhart[18].

Several possible functions for the germ line limited DNA sequences have been proposed (see Ammermann[2] and Tobler[45] for review): The extra chromatin in the germ line might be important for 1) the development of germ cells and/or for gamete formation, 2) it could be necessary for the control of gene expression by e.g. suppressing the activity of genes which are active only in somatic cells, 3) could have important functions in meiotic processes such as chromosome pairing and recombinational events, 4) might increase the volume of germ line nuclei and by doing so slow down the duration of the cell cycle, 5) might be needed for continuous mitotic cell divisions in the germ line since nematodes are cell-constant animals, 6) might represent a genetic reservoir for evolutionary purposes, and 7) might have no function at all and consist of useless, junk or selfish DNA. Moreover, it has specifically been suggested that chromatin diminution in *Ascaris* might serve the purpose of reducing the number of ribosomal genes that might have selectively been amplified[24] or carried along as independent rDNA episomes in the germ line[46]. Finally, the cast-off chromatin might be converted to ribose nucleotides needed for rapid growth of somatic cells[34].

In order to analyze the process of chromatin diminution as well as the composition and informational content of the eliminated DNA sequences at the molecular level, we initiated about 15 years ago a molecular biological study of germ line and somatic DNA in *Ascaris lumbricoides* var. *suum*[41]. This parasitic nematode was used for our experiments rather than the horse parasite *Ascaris megalocephala*, because the latter is unfortunately no longer readily available. *Ascaris megalocephala*, the species chosen by Boveri[8–11] for his classical analysis of chromatin

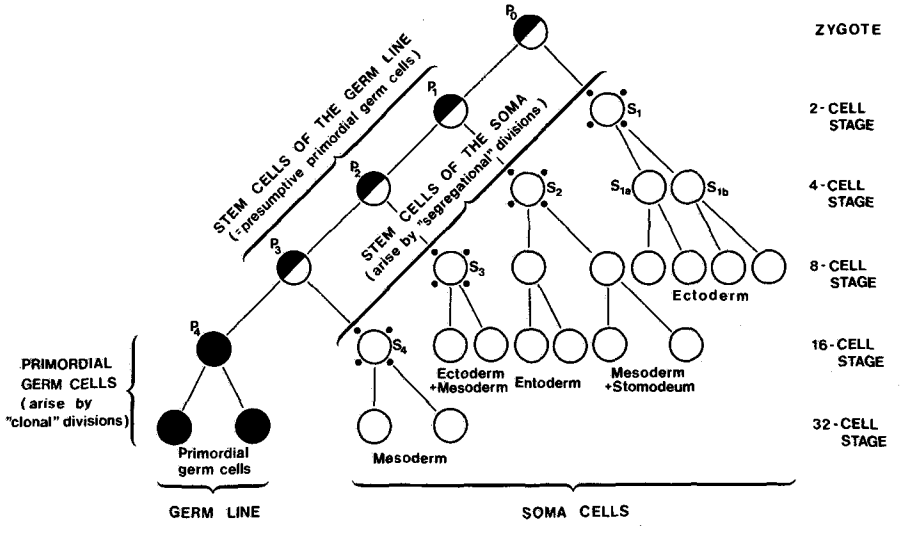

Figure 2. Schematic representation of the segregation of germ line and somatic cells in *Ascaris megalocephala* (modified after Boveri[11]). The presomatic cells S_1–S_4 undergoing chromatin diminution are indicated by ♀. Final segregation of germ and somatic cell lines is achieved in the 16-cell stage where the P_4 cell gives rise only to germ line cells.

Occurrence of chromatin diminution in nematodes. The second column shows the developmental stages at which chromatin diminution takes place; the question marks indicate that such stages have either not been established or remain uncertain

Species	Developmental stage (cleavage division)	Reference
Parascaris equorum *(= A. megalocephala)*	2nd or 3rd to 5th	Boveri, 1887, 1910[8,11]
Ascaris lumbricoides var. *suum* *(= A. lumbricoides)*	3rd to 5th	Meyer, 1895[26]; Bonnevie, 1902[7]
Ophidascaris filaria *(= A. rubicunda)*	2nd or 3rd to 5th	Meyer, 1895[26]
A. anguillae *(= A. labiata)*	?	Meyer, 1895[26]
Contracaecum incurvum *(= A. incurva)*	3rd to 5th (?)	Goodrich, 1916[20]
Toxocara canis *(A. canis)*	2nd to 6th	Walton, 1917, 1924[47,48]
T. cati *(= Belascaris mystax)*	2nd or 3rd to 6th	Walton, 1924[48]
T. vulpis *(= Belascaris triquetra)*	3rd to 6th	Walton, 1924[48]
Cosmocerca sp.	3rd to 8th (?)	Yao and Pai, 1942[51]
Physaloptera indiana	1st to 3rd	Goswami, 1973[21]
*Strongyloides papillosus**	During mitotic parthenogenesis giving rise to free-living ♂	Albertson et al., 1979[1]

*This species does not show the typical elimination of chromosomal material in presomatic cells and represents therefore a special case of chromosome elimination.

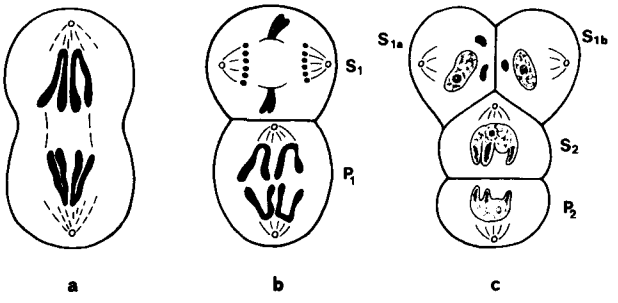

Figure 3. Chromatin diminution in the parasitic nematode *Ascaris megalocephala*[44] (after Boveri[10]). *a* First cleavage division, *b* second cleavage division. Chromatin diminution takes place in the top S_1 cell but not in the lower P_1 cell. *c* 4-cell stage after completion of the second cleavage division. The cells S_{1a}, S_{1b} and S_2 give rise exclusively to somatic cells, whereas the P_2 cell represents the stem cell of the germ line. The cast-off chromatin is clearly visible in the cytoplasm of the presomatic cells S_{1a} and S_{1b}.

diminution, has the advantage of possessing large, up to about 20 µm long chromosomes in the germ line cells, whereas *Ascaris lumbricoides* has 48 tiny chromosomes in the diploid female and 43 in the diploid male germ line cells[15,29,17]. However, since chromatin diminution takes place during the second or third cleavage divisions in *Ascaris megalocephala*[8], but never before the third cleavage division in *Ascaris lumbricoides*[7,26], in the latter species isolation of prediminution DNA from early cleavage stages is easier, because 4-cell stages contain more undiminished DNA than 2-cell stages[41].

The following important questions need to be answered if the cause of chromatin diminution and the significance of the germ line limited DNA sequences are to be understood. 1) Why does chromatin diminution and chromosome elimination take place in certain species? 2) How much DNA is eliminated? 3) What is the genetic informational content and the function of the eliminated DNA sequences? 4) How does chromatin elimination occur; what is the mechanism of DNA loss at the molecular level? We believe that an understanding of the significance of chromatin diminution and the germ line limited DNA sequences depends on the prior elucidation of the genetic informational content of the eliminated DNA sequences as well as on the understanding of the mechanism of the elimination process.

Amount of eliminated DNA in Ascaris

The amount of eliminated DNA can easily be determined by comparing the genome size before and after chromatin diminution. This was first done in *Ascaris lumbricoides* using the isotope dilution technique[41]. A genome size of 0.63 pg or 5.8×10^8 bp was established for the haploid germ line cells and of 0.46 pg or 4.2×10^8 bp for the haploid somatic cells, respectively. The eliminated DNA therefore amounts to 0.17 pg or 1.6×10^8 bp, or in other words to 27% of the total germ line genome[41]. Later figures from three different laboratories[19,28,35], ranging from 22% to 34% eliminated DNA in *Ascaris lumbricoides*, agree quite well with our original value. There is one group which reported that the eliminated DNA amounts to as much as 56%[13]. The percentage of eliminated DNA has been established by using different techniques such as renaturation kinetics[19], diphenylamine tests[13,28], Feulgen-microspectrophotometry[28,35] and isotope dilution[13]. It may be added that the horse parasite *Ascaris megalocephala* seems to eliminate much more DNA, namely roughly 85%[27,28].

Characterization of the germ line and somatic genome of Ascaris lumbricoides *by renaturation kinetics*

In our original study of the eliminated DNA sequences in *Ascaris lumbricoides*[41], we analyzed the reassociation kinetics of DNA from germ line and somatic cells. DNA isolated from spermatids and embryonic 4-cell stages was used as a source for germ line DNA sequences, and DNA from 12-day-old moving larvae as a source for somatic DNA, respectively. The results are presented in figure 4. About 10% of the retained somatic DNA sequences are repetitious with an average family size of about 6000 copies, the rest appears to consist of unique sequences. However, germ line DNA from spermatids contains about 23% fast-renaturing DNA sequences repeated about 7000–10,000 times in the germ line genome. Since 27% of germ line DNA is expelled from presumptive somatic nuclei during chromatin diminution, we had to conclude that the eliminated DNA consists of repetitive and unique sequences in a ratio of approximately 1:1[41]. In 4-cell stages, the fraction of highly repetitious DNA sequences is increased by about 25% as compared to spermatid DNA. Density gradient centrifugation and electron microscopy revealed that 4-cell stages of *Ascaris*

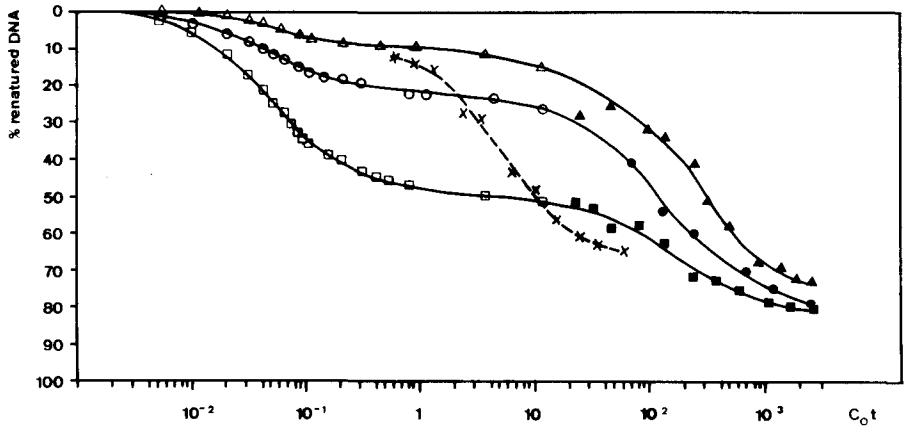

Figure 4. Renaturation kinetics of DNA from *Ascaris lumbricoides*[41]. All DNAs were sheared, heat denatured, incubated at 60 °C in 0.12 M PB 0.001 M EDTA, and their rate of renaturation determined. C_0t points up to a C_0t of 10 were determined in a spectrophotometer (open symbols). The DNAs were reassociated at concentrations of approximately 50 µg/ml. C_0t points above 10 were determined by hydroxyapatite binding (filled symbols). The values were corrected for partially reannealed sequences according to Britten and Kohne[45]. Larval DNA (▲—▲) was reassociated at 400 µg/ml, spermatid DNA (●—●) was reassociated at 200 µg/ml and 4-cell stage DNA (■—■) was reassociated at 165 µg/ml. The dashed line represents the renaturation of ^{32}P-labeled *Escherichia coli* DNA (conc. approx. 10 µg/ml).

lumbricoides contain about 25–40% mitochondrial DNA[41,42]. Since DNA has been isolated from whole 4-cell stages and not from a nuclear preparation, the presence of a sizable fraction of mitochondrial DNA was to be expected in this DNA preparation[41].

Moritz and Roth[28], using slightly different techniques and methodology, came to the conclusion that only highly repetitious DNA sequences are eliminated during chromatin diminution in *Ascaris lumbricoides*. In an attempt to resolve this conflicting situation, Goldstein and Straus[19] repeated the reassociation kinetic experiments and reported that the eliminated DNA sequences in *Ascaris lumbricoides* consist of repetitive and unique sequences in a ratio of approximately 1:1, thus confirming our earlier findings and conclusions. There is the possibility that the differences between the results of Moritz's group[28,36,37] on the one hand and Goldstein and Straus[19] and ours[41] on the other, may have arisen from real differences between presumed subspecies of *Ascaris lumbricoides*[19]. Our *Ascaris* and that of Goldstein and Straus[19] are of North American origin, while those of Moritz's group[28,36,37] are from Europe. However, since the domesticated pig has been brought to North America by man in recent time, and in all likelihood with it concomitantly its intestinal parasite *Ascaris lumbricoides* var. *suum*, one would not expect the two varieties of *Ascaris* to be genetically very different. As things stand right now, we favor the interpretation given by Roth[36], who also found 10% of intermediate repetitive DNA sequences in the soma of *Ascaris lumbricoides* and who thereby offered a plausible explanation of the seemingly conflicting results and interpretations.

The repetitious DNA sequences of germ line and somatic cells are qualitatively different

Since in *Ascaris lumbricoides* germ line cells clearly contain more repetitive DNA sequences than somatic cells, the important question arose, whether the germ line limited repetitive DNA sequences are qualitatively or merely quantitatively different from the repetitive DNA sequences retained in the somatic cell lines. In order to tackle this problem, sequential hybridization experiments were performed[41] (cf. fig. 5). At the time these experiments were done, radioactively labeled DNA from *Ascaris* was not readily available. We therefore used complementary RNA (cRNA) derived from spermatid (germ line) and larval (soma) DNA in our hybridization experiments. The two cRNAs were hybridized in increasing amounts to either spermatid or larval DNA. As figure 5a demonstrates, larval cRNA at saturation anneals to about 11% of the spermatid DNA, and spermatid cRNA to about twice as much. If saturating amounts of larval cRNA are hybridized together with increasing amounts of spermatid cRNA, the extent of hybridization rises again to reach the same saturation level as with spermatid cRNA alone. This result clearly indicates that germ line DNA does contain sequences which are no longer present in larval DNA. However, since filter hybridizations and low Cot conditions were used, only the repetitious sequences of the *Ascaris* genome have been analyzed in these experiments. Therefore, the above experiment allows only the conclusion that the eliminated repetitious sequences are distinct from the repetitious sequences retained in somatic cells. The same conclusion has been reached by Roth[36]. In control experiments, larval or spermatid cRNA was annealed to larval DNA (fig. 5b). As was to be expected, the saturation plateaus are the same for both cRNAs. Moreover, the saturation level is not changed by the addition of increasing amounts of spermatid cRNA to saturating amounts of larval cRNA or vice versa. This result is to be expected, since all somatic DNA sequences must of course be contained in the genome of the germ line cells.

Germ line and somatic cells contain the same percentage of genes coding for 18S and 28S rRNA

It has been proposed that chromatin diminution serves the purpose of discarding large amounts of rRNA genes that might have selectively been amplified during oogenesis or following fertilization[24], or which persist as independent rDNA episomes in the germ line[46]. In order to test this hypothesis, saturation hybridization experiments

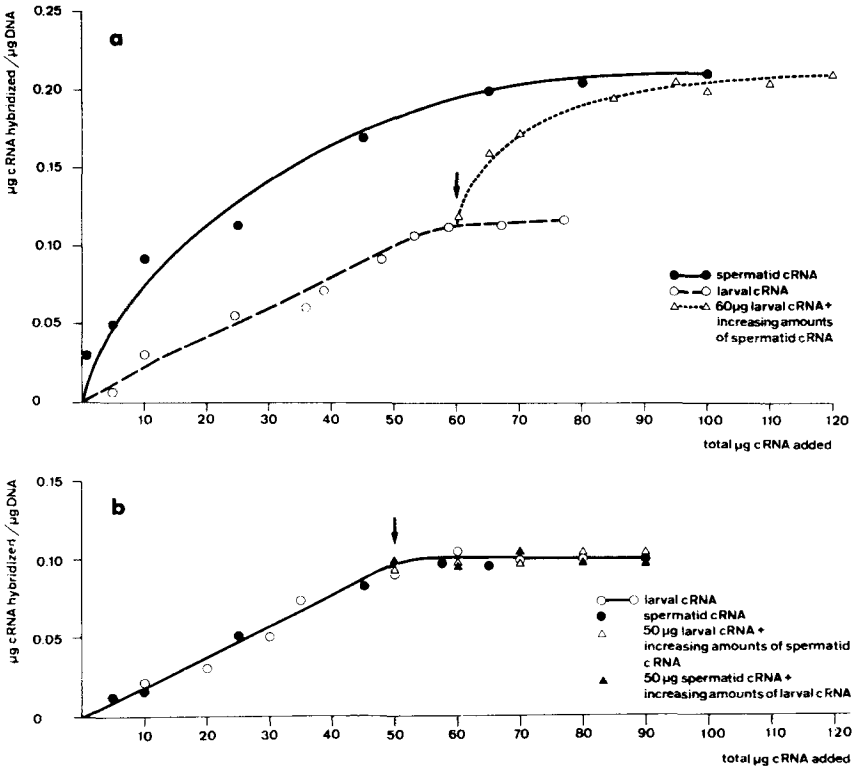

Figure 5. *a* Saturation of *Ascaris* spermatid DNA with increasing amounts of larval (○—○) and spermatid (●—●) cRNA. After the saturation value for larval cRNA was determined (60 µg/larval cRNA), increasing amounts of spermatid cRNA (△···△) were hybridized in the presence of saturating amounts of larval cRNA. *b* Saturation of *Ascaris* larval DNA with increasing amounts of larval (○—○) and spermatid (●) cRNA. In additional experiments, saturating amounts of larval cRNA in the presence of increasing amounts of spermatid cRNA (△) or saturating amounts of spermatid cRNA with increasing amounts of larval cRNA (▲) were hybridized to larval DNA[41].

of 18S and 28S rRNA with both germ line and somatic DNA were carried out. The results clearly show that no quantitative elimination of ribosomal genes takes place during chromatin diminution in *Ascaris lumbricoides*[41,43]. Recently, we have analyzed the structural organization of the ribosomal genes in *Ascaris lumbricoides* in much more detail and found that the rDNA cluster of *Ascaris lumbricoides* exists in two main size classes of 8.8 kb and 8.4 kb, the length heterogeneity being due to a 450 bp long insertion located in the non-transcribed spacer region of the larger rDNA unit[3]. The quantitative ratio of the two rDNA size classes is on the average roughly 10:1 in the investigated wild-type population of *Ascaris lumbricoides*, but varies to a great extent between different individuals[4]. However, since no differences have been detected in the hybridization pattern of the two rDNAs between germ line and somatic cells in any single individual tested, one can further conclude that chromatin diminution does not qualitatively change the rDNA pattern of germ line versus somatic cells[4].

The germ line limited chromatin contains a large amount of satellite DNA which is not needed in somatic cells

Moritz and Roth[28] were the first to report the presence of a highly repetitive DNA fraction with satellite DNA properties in *Ascaris lumbricoides* and *Parascaris equorum* (= *A. megalocephala*). They later on succeeded in preparatively isolating an AT-rich DNA satellite from the germ line of *Ascaris lumbricoides*[37] and characterized the DNA satellite by restriction enzyme analysis. They also reported that the germ line limited satellite DNA is composed entirely of two related families of repeated sequences, one repeating unit being 125 bp, the other one 131 bp long[37]. The germ line contains about 5×10^5 copies of repeating units, but a limited number of copies is also retained in the somatic cells[37]. Sequencing studies[39] revealed that the originally communicated length values of the prototype sequences had been overestimated and demonstrated that both AT-rich variants are actually only 123 bp long. Furthermore, they have shown that the two prototype sequences differ in about 20% of their base sequence[39].

Independently of Moritz's group, we have carried out a similar analysis on the structural organization of the satellite DNA contained in the eliminated genome of *Ascaris lumbricoides*, thus confirming and extending several of their results[30,31]. Since they used uncloned material for their experiments, the reported prototype sequence is representative for the major portion of the satellite DNA. However, this method does not allow a demonstration of the diversity between and within different variant classes. We have isolated, cloned and sequenced several restriction endonuclease fragments derived from the germ line DNA satellite[30,31]. A comparison of all the determined sequences, which differ only by small deletions, insertions and single base substitutions, allowed the establishment of a consensus sequence[31] (cf. fig. 6). This sequence is in good agreement with those published by Streek et al.[39]. One of their prototype sequences is very similar but not identical with our consensus sequence, the other one can be arranged among our Bam fragments[31].

The comparative analysis of our 121 bp long consensus sequence has provided much evidence for an internal short range periodicity of 11 bp length[31]. The deduced subrepeat is an undecanucleotide with the sequence 5'GCA($^{T}_{A}$)TT($^{T}_{G}$)TGAT[31]. It is therefore likely that the *Ascaris* satellite has evolved from an ancestral variant of this 11 bp long prototype sequence.

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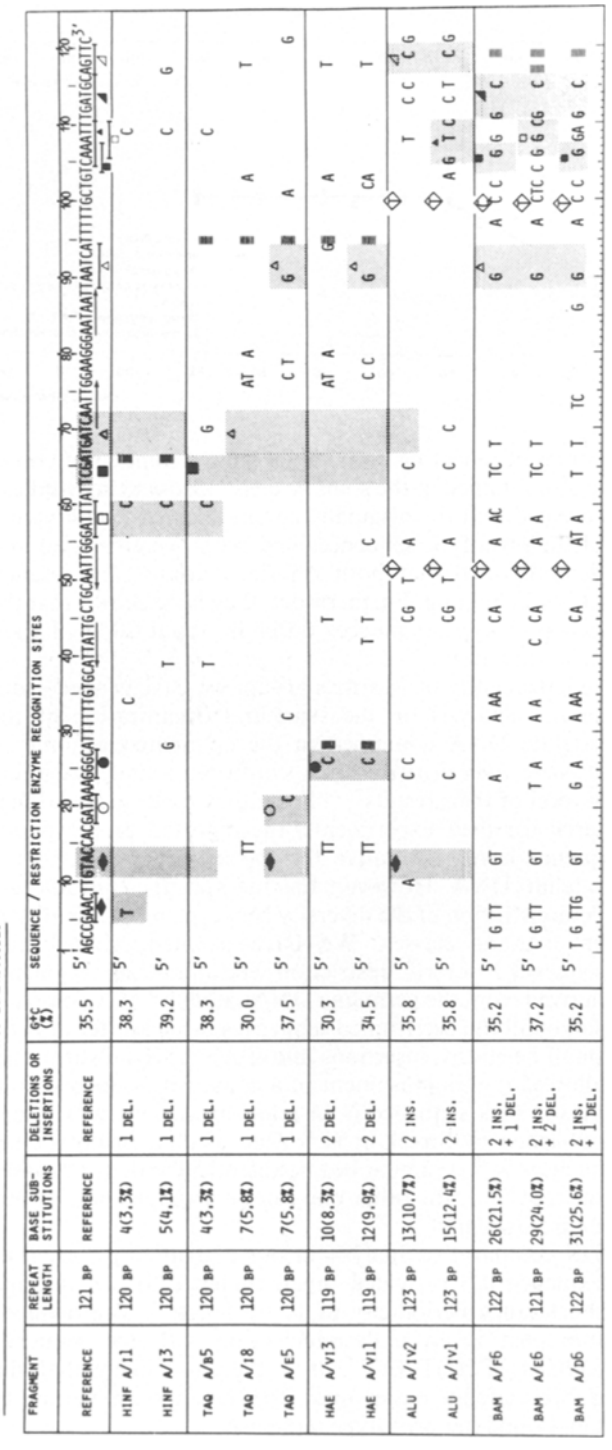

Figure 6. Consensus sequence (on top) and sequences of 12 individual clones of highly repetitive germ line DNA of *Ascaris lumbricoides*. The reference sequence represents the consensus sequence deduced from all sequenced DNA fragments. The different sequences are listed in order of decreasing homology. Base substitutions relative to the reference sequence are indicated by the respective bases, deletions by ≡ and insertions by ◇. Arrows underlining the reference sequence designate small palindromic sequences. The restriction enzyme recognition sites are ♦ Rsa I, ○ Mbo I, ● Hae III, □ Hinf I, ■ Taq I, △ Bcl I, ▲ Eco RI, ◥ Bam HI, ◿ Alu I; they are indicated by stippled areas[31].

In order to determine the amount of eliminated satellite DNA during chromatin diminution, saturation hybridization experiments of germ line and somatic DNA with labeled monomeric satellite fragments were performed[31] (cf. fig. 7). The difference in the two saturation values clearly demonstrates that chromatin diminution removes over 99.5% of the satellite DNA sequences from the presumptive somatic cells. Since we estimate the satellite to represent about 20% of the germ line genome, corresponding to about 10^6 copies of the 120 bp repeating units[31], somatic DNA would at most contain roughly 5000 copies. For our saturation hybridization experiment presented in figure 7, an internal control standard was used. The same filters which were hybridized with a satellite DNA probe, were also used for hybridization with cloned 18S and 28S rDNA from *Ascaris lumbricoides*. As figure 7 clearly shows, the saturation values for rDNA are almost identical for germ line and soma DNA.

Since in *Ascaris lumbricoides* development and differentiation of the somatic cell lines proceed normally after chromatin diminution in the presomatic cells has taken place, one has to conclude that the germ line limited DNA sequences are not needed for the normal function of somatic cells and tissues.

Satellite DNA is not transcribed

The question whether or not satellite DNA sequences are transcribed in different *Ascaris* tissues has been investigated in the following way: Total nuclear RNAs were first isolated from larvae, intestines, spermatids, oogonies and oocytes. These different nuclear RNAs, 18S and 28S rRNA, pBR 322/HinfI DNA and a cloned monomer BamF6 satellite DNA fragment as internal standard were denatured, separated on an agarose gel, transferred to DBM paper and hybridized with labeled Taq I and Bam HI cloned monomers. Although hybridization with the internal standard, which corresponds to 0.025% of the nuclear RNA in each slot, gives a clear signal (cf. fig. 8), there is no hybridization with 18S and 28S rRNA or with any nuclear RNA tested. In the reciprocal experiment, labeled nuclear RNAs extracted from different tissues and stages were hybridized to filter-bound satellite DNA. Again, there was no hybridization signal detectable. The conclusion therefore is that satellite DNA sequences of *Ascaris lumbricoides* are either not transcribed in any of the tested cells, or that the transcripts are so few that they were not detected by the experimental procedures applied.

Turning now to the possible function of the eliminated *Ascaris* satellite DNA, the available data are consistent with the notion that satellite DNA exerts its biological effects in processes that are inherent to the germ line cells. Therefore, if this satellite DNA has any function at all, it must be germ line limited. Otherwise one would have to assume that the same function could also be exerted by the few satellite DNA copies remaining in the somatic cells, which is however rather unlikely.

The germ line limited chromatin contains also DNA sequences other than satellite DNA

We have already shown that the eliminated chromatin contains a large amount of satellite DNA sequences. The

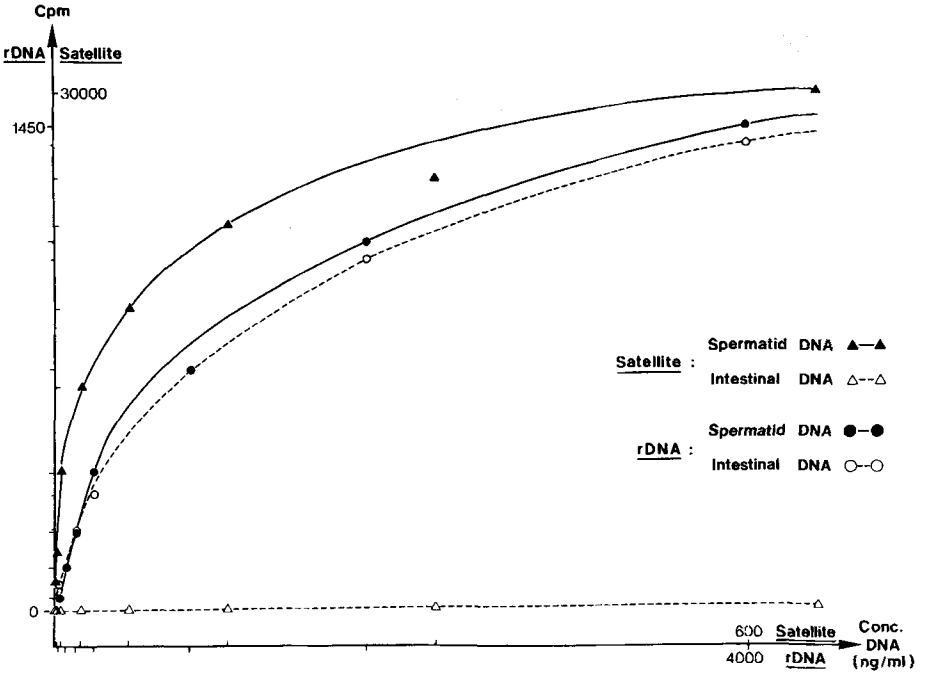

Figure 7. Saturation hybridization of a Mbo I monomeric satellite DNA fragment to spermatid (▲—▲) and intestinal (△—△) DNA of *Ascaris lumbricoides*. As an internal control, ^{32}P-labeled cloned 18S and 28S rDNA from *Ascaris lumbricoides* were hybridized to the same filters loaded either with 30 ng of spermatid (●—●) or with 30 ng of intestinal (○—○) DNA[31].

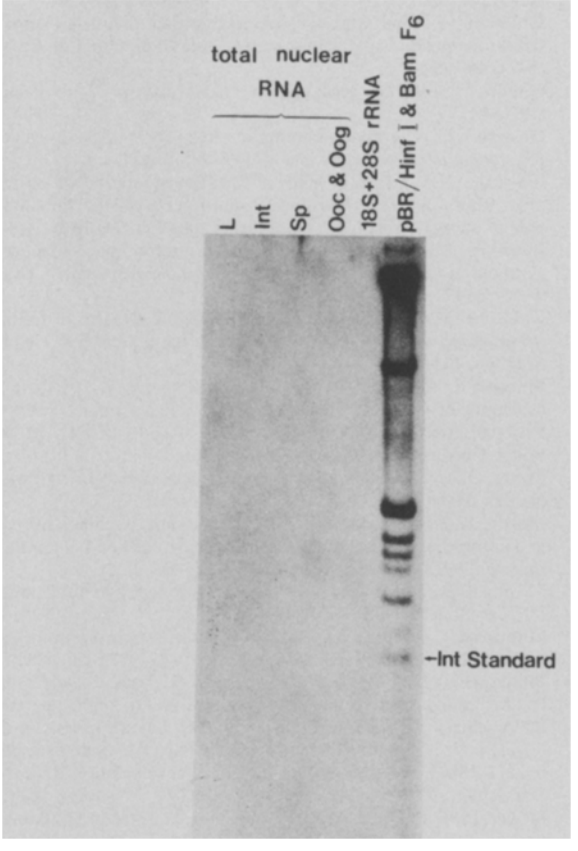

Figure 8. Total nuclear RNAs were isolated from larvae (L), intestines (Int), spermatids (Sp), oocytes (Ooc) and oogonies (Oog) of *Ascaris lumbricoides*. 5 µg aliquots of these different RNAs, as well as 0.5 µg of 18S and 28S rRNA, 50 ng of pBR 322/Hinf I together with 50 ng of the clone Bam F6 (corresponding roughly to 1.25 ng of the cloned Bam HI monomer) as an internal standard were run on a 2% agarose slab gel, transferred to DBM paper and hybridized with labeled Taq I and Bam F cloned monomers. Hybridization of the labeled probe is only detectable with the internal standard[30].

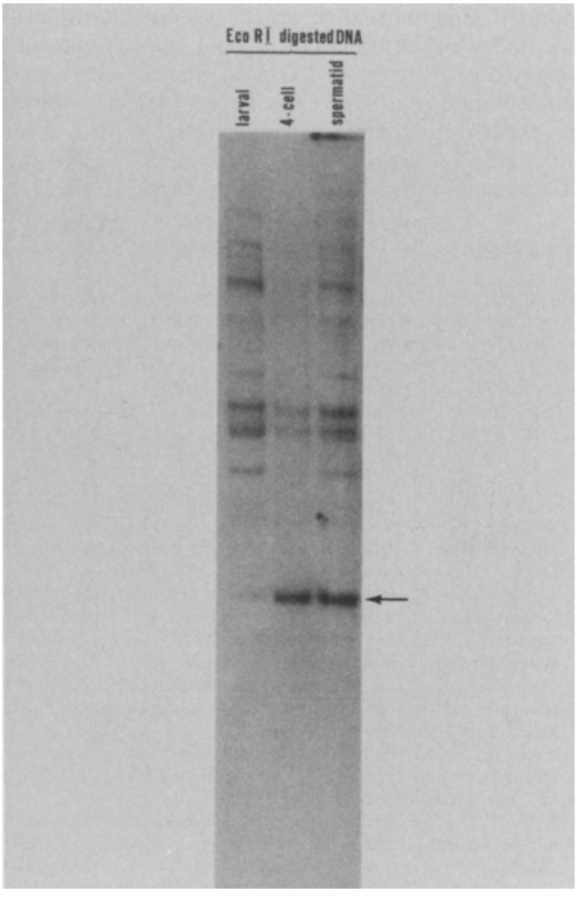

Figure 9. Hybridization pattern of EcoRI digested total DNA from larvae, 4-cell stages and spermatids of *Ascaris lumbricoides*. The restriction fragments were separated on an agarose slab gel, Southern transferred and hybridized with a labeled subcloned DNA fragment originally arising from the *Ascaris* germ line gene library. The arrow points to a 1 kb band which is prominent in the germ line but severely reduced in the somatic genome.

important question of course remains whether other DNA sequences apart from the satellite DNA are also expelled from presumptive somatic cells. In order to solve this problem, a gene library of *Ascaris lumbricoides* germ line DNA was first established, using the in vitro packaging system with the phage λ 1059 as vector. Such a gene library enabled us to pick out several clones which comprise both satellite and nonsatellite DNA sequences present in the *Ascaris* germ line genome. One of these clones has been further analyzed. A physical map was constructed and a 6 kb long fragment containing the junction between satellite and nonsatellite DNA was subcloned in pBR 322. This pBR subclone was further subdivided into smaller DNA fragments which were again cloned, yielding pure hybridization probes for the study of their behavior during chromatin diminution. An example of this strategy is presented in figure 9. A labeled subcloned DNA fragment derived from the *Ascaris* germ line gene library and containing no satellite DNA sequences hybridizes clearly to a Southern transfer of Eco RI digested germ line and somatic DNA. However, whereas the hybridization patterns for 1–10-kb-long DNA fragments are almost identical for the germ line and somatic genome, this is not true for the 1-kb-long band (arrow in fig. 9). Clearly, the hybridization signal is much stronger with the germ line than with the somatic genome. Using internal standards, we have been able to show that the 1-kb band in the germ line genome corresponds to roughly 50–100 copies, whereas the somatic genome contains at most three copies (data not shown). This experiment therefore demonstrates quite convincingly that DNA sequences other than satellite DNA must be contained in the germ line limited chromatin and therefore be expelled from the presumptive somatic cells during the process of chromatin diminution.

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Short Communications

Uninterrupted protein synthesis is essential for survival in the early stages of carbontetrachloride-induced hepatocellular necrosis in the mouse

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Summary. The fatal syndrome produced by cycloheximide given 6 h after a hepatonecrogenic dose of CCl_4 is due neither to direct toxic synergism between CCl_4 and cycloheximide nor to transient sinusoidal thrombosis. It is suggested that survival in the presence of unknown factors released from dying liver cells requires uninterrupted protein synthesis. The life-saving effect of sterilization of the intestine by antibiotics indicates that the gut flora or its products play a vital role in pathogenesis.
Key words. CCl_4; hepatocellular necrosis; protein synthesis inhibition; Ancrod; antibiotics; gut flora.

A lethal, shock-like syndrome occurs when mice given a hepatonecrogenic dose of CCl_4 are challenged 6 h later (but not if challenged 18 h later) with a dose of 30 µg/g b.wt of cycloheximide[2]. It was shown that heparin protected against these fatalities, leading to the tentative hypothesis that transient centrilobular obstruction by sinusoidal thrombosis led to irreversible ischaemic damage to the midgut[2]. Cycloheximide was thought to act by preventing protein synthesis, suggested by other work[3] to be essential for the physiological release of endogenous anticoagulant heparin.

The present report extends these initial observations, shows that thrombosis plays no essential part in the evolution of this lethal syndrome, and indicates a more complex pathogenesis involving the process of hepatocellular necrosis and the gut flora or its products.

Materials and methods. Male, inbred CBA-strain mice, 20–25 g in weight were used, and allowed free access to food and drink throughout.

Carbon tetrachloride (Analar grade, B.D.H. Ltd, England) was mixed with an equal volume of olive oil (B.P.), and 0.1 ml of the mixture given by s.c. injection. Prior observation confirmed that this dose caused centrilobular hepatic necrosis but no detectable renal tubular necrosis. Cycloheximide (Sigma Chemical Co. Ltd, England) was dissolved in sterile 0.9% aqueos NaCl solution to give a concentration of 30 µg/0.01 ml, and given i.p. as a dose of 30 µg/g b.wt. This dosage gives approximately 90% inhibition of protein synthesis as measured in the mouse liver[4], and is tolerated by all normal mice. Ancrod, 70 U/ml (Armour Pharmaceutical Co. Ltd, England), was diluted with sterile saline to give a concentration of 0.05 U/0.01 ml, and injected i.p. as a dose of 0.05 U/g b.wt. At this dose level, Ancrod produces complete defibrination in the mouse (personal communication from Berk Pharmaceuticals, Ltd, England). Neomycin sulphate and bacitracin (Sigma Chemical Co., Ltd, England), 4.0 g of each were dissolved in 1.0 l of boiled, cooled tap water and the solution brought to pH 4.0 by addition of 1.0 N HCl. This antibiotic solution was substituted for drinking water for 4 full days before start of the relevant experiment as recommended by van der Waaij and Sturm[5], and replaced by tap water 48 h after the start of the experiment.

All injections were given under light ether anesthesia. Moribund mice were killed by cervical dislocation under ether anesthesia; such animals, together with mice dying during the course of the experiments, were necropsied and major organs sampled for routine histological examination.

Statistical analysis of the results was by the Fisher-Irwin exact probability test (two-sides).

Experiment 1. Three groups each of five mice were used. Group A mice were given Ancrod followed immediately by CCl_4; 6 h later the mice received cycloheximide. Group B mice received