Trichinella spiralis-Infected Muscle Cells: Abundant RNA Polymerase II in Nuclear Speckle Domains Colocalizes with Nuclear Antigens

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Infection of mammalian skeletal muscle cells by Trichinella spiralis causes host nuclei to become polyploid (ca. 4N) and abnormally enlarged. It has been postulated that this enlargement reflects an infection-induced elevation of host transcription. Anthelmintic treatment of T. spiralis-infected rodents with mebendazole (MBZ) causes a reduction in the size of infected cell nuclei and a significant reduction in the total RNA content of individual infected muscle cells. A monoclonal antibody to the large subunit of RNA polymerase II (Pol II) was used here to assess the effects of infection on Pol II levels in isolated infected cell nuclei. Pol II was localized to speckle domains in isolated infected cell nuclei. Similar domains have been previously localized to sites of RNA synthesis or processing. When compared to the levels in nuclei from other, uninfected host cells, speckle-localized Pol II (SL-Pol II) levels were significantly elevated in infected cell nuclei by a mean of 3.9- to 6.8-fold. Nuclear antigens (NA) recognized by antibodies against T. spiralis localized to infected cell nuclei. By use of confocal microscopy, a subpopulation of NA was found colocalized with most speckle domains defined by Pol II. MBZ treatment of chronically infected mice, which depletes NA from infected cell nuclei, caused a significant depletion of SL-Pol II from infected cell nuclei. Control nuclei had a mean of 70% more SL-Pol II than MBZ-treated nuclei. The mean residual level of Pol II in these polyploid nuclei remained elevated by 120% over the level in 2N control nuclei. These observations may indicate two distinct effects of infection on Pol II levels in host cells.

Trichinella spiralis is an intracellular parasite of mammalian skeletal muscle cells during early larval development. Infection by the parasite causes a permanent phenotypic reprogramming of these host cells. Some major features of the affected host cells include (reviewed in references 5 and 10) (i) infection-induced reentry into the cell cycle by 5 days postinfection (dpi); (ii) chronic suspension in apparent G0/M of the cell cycle; (iii) chronic repression of muscle gene expression; (iv) expression of an infected cell phenotype that remains ill defined; (v) nuclei and nucleoli that exhibit extraordinary enlargement; and (vi) acquisition of a pronucleon collagen capsule.

Specific changes in host cell nuclei establish a potential genetic explanation for at least some chronically infected cell characteristics. For instance, the levels of transcripts for the muscle transcription factors MyoD and myogenin were significantly reduced in infected muscle cells (11), demonstrating the repression of muscle gene transcription. In addition, the levels of transcripts and proteins for myofibrillar contractile elements were significantly reduced in these cells (11, 12). In contrast, the levels of transcripts for host collagen isoforms and vascular endothelial cell growth factor were significantly elevated during the infection (3, 20). These results indicated that the infection causes a redirection of host gene expression.

It is possible that displacement of the host muscle cell out of G0 accounts for muscle gene repression (10, 11), since muscle gene activation is normally restricted to G0/G1 (19). However, based on current knowledge, the elevated level of collagen isoform expression is not readily explained by the same argument. Elevated levels of other host cell products, e.g., acid phosphatase activity and nuclear lamins, also occur (13, 17, 26). Furthermore, the content of lamins A and C in infected cell nuclei was found to be over sixfold higher than that in 2N nuclei from other host cells (26). Hence, the infected cell phenotype might reflect the repression of muscle genes, the abnormal activation of other genes, and additional enhancement in the expression of host proteins beyond levels normally encountered.

Infected cell nuclei and nucleoli are abnormally enlarged. It was suggested that this enlargement could reflect transcription elevated by the parasite in infected cells (9). For instance, antigens ranging in size from about 71 to 97 kDa were detected in host cell nuclei by antibodies specific to a parasite-encoded glycan determinant (14, 26, 27). These antigens are referred to as nuclear antigens (NA). NA become apparent in infected cell nuclei by 9 dpi and chronically persist in these nuclei for the duration of the infection (6). NA colocalize with host chromatin in the nucleoplasm, exclusive of nucleoli or the nucleoskeleton. NA can be depleted from host cell nuclei by the anthelmintic agent mebendazole (MBZ). NA depletion preceded a diminution in the size of infected cell nuclei and nucleoli (26). The greatest change in nuclear morphology occurred following 8 days of treatment, after which a plateau was reached. Mean levels of total RNA, protein, and acid phosphatase activity

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were reduced by about half in these infected cells, when measured after the plateau was reached. The levels of host nuclear lamins were also significantly reduced in infected cell nuclei following MBZ treatment. Significantly, all of these MBZ-induced effects were specific to infected cells. Collectively, these observations are consistent with a parasite-mediated effect on host gene expression, which may include enhancement of host cell transcription.

Since NA have been detected in the nucleoplasm but not in nucleoli, an influence of the parasite on host gene expression can best be rationalized for host transcription mediated by RNA polymerase II (Pol II), rather than RNA polymerase I. However, attempts to directly measure transcription rates in infected cell nuclei have been unsuccessful. As an alternative approach, immunostaining was used here to assess Pol II levels in infected cell nuclei. Pol II occurs in both diffuse and discrete nucleoplasmic compartments (2). The diffuse components are extractable by nonionic detergents, whereas the discrete compartments are not extractable by detergents and are called speckles. Nuclear speckles have been associated with RNA synthesis and processing (reviewed in reference 7). The large subunit of Pol II occurs in both hyper- and hypophosphorylated isoforms. Hyperphosphorylated Pol II has been localized in speckle domains (18). Monoclonal antibody (MAb) 8WG16 recognizes multiple phosphorylated isoforms of Pol II, including Pol II 0, and binds to nuclear speckles in detergent-extracted nuclei (2, 24). Using MAb 8WG16, we evaluated speckle-localized Pol II (SL-Pol II) in infected cell nuclei. Our findings introduce the possibility that SL-Pol II levels are elevated in infected cell nuclei by a multistep process, one step of which may involve chronic input from the parasite. We further show that NA colocalize with speckle domains of infected cell nuclei.

MATERIALS AND METHODS

Parasites and animals. The strain of T. spiralis was originally obtained from Dickson Despommier (Columbia University) and was maintained by serial passage in BALB/c mice as previously described (12). BALB/c mice and rats used in this research were obtained from the Bantust Vavarium (Washington State University) and Simonsen Laboratories, Gilroy, Calif., respectively. Mouse and rat infections were initiated by oral inoculation of 750 and 10,000 muscle larvae, respectively. Animals used in this investigation carried chronic infections for 2 to 8 months postinfection.

For depletion of NA and Pol II from host cell nuclei, BALB/c mice harboring chronic infection with T. spiralis were treated daily with either MBZ (100 mg/kg) dissolved in corn oil (25 mg ml−1) or corn oil alone. These mice also received injections of cyclophosphamide at the beginning of the experiment and at 7 dpi, as previously described (26), to reduce host inflammation resulting from MBZ treatment. Infected cell nuclei isolated from these animals were used in immunoblotting and antibody staining assays.

Antibodies. MAb 9D4 (rat immunoglobulin G1 [IgG1]) was generously provided by J. A. Appleton (Cornell University). This MAb is specific to a tyvelose-sylated p43 antibodies (8). a-DG p43 are indistinguishable (27). Two control peptides were also used. One consisted of 24 amino acid residues (SAAPSTPAAPSPPQRPAETQQTQD) from a Babesia bovis variable merozoite surface antigen (15). The other consisted of 30 amino acid sequence PTSPSYS is a consensus sequence of a degenerate repeat in the C terminus of Pol II. This consensus sequence is recognized by MAb 8WG16 (22). A peptide containing three contiguous PTSPSYS motifs was synthesized, purified by high-pressure liquid chromatography (Genemed Synthesis, Inc., San Francisco, Calif.; 96.7% purity), and used in antibody inhibition experiments. Two control peptides were also used. One consisted of 24 amino acid residues (SAAPSTPAAPSPORRPAETQQTQD) from a Babesia bovis 44-kDa variable merozoite surface antigen (15). The other consisted of 30 amino acid residues (SSSDSDEGEEITLPEDGVEEPDDVQIL) from a B. bovis 225-kDa spherical body protein (16). All peptides were dissolved in phosphate-buffered saline (pH 7.4) at a final concentration of 1 mg/ml. For peptide inhibition

FIG. 1. Immunoblot using MAb 8WG16. Extracts of infected cell nuclei (5 × 106), isolated as described in Materials and Methods, were probed by immunoblotting using MAb 8WG16 (lane 1) or an isotype control MAb (IgG2a) (lane 2), each at 10 µg ml−1. The label on the right indicates bands at estimated 210 and 240 kDa which coincide with major hypo- and hyperphosphorylated forms of the large subunit of Pol II. The dashes on the left indicate size standards in the descending order of 220, 97.4, 66, 46, 30, 21.7, and 14.3 kDa.

Inhibition of MAb 8WG16 binding by the Pol II hexapeptide repeat. The amino acid sequence PTSPSYS is a consensus sequence of a degenerate repeat found in the C terminus of Pol II. This consensus sequence is recognized by MAb 8WG16 (22). A peptide containing three contiguous PTSPSYS motifs was synthesized, purified by high-pressure liquid chromatography (Genemed Synthesis, Inc., San Francisco, Calif.; 96.7% purity), and used in antibody inhibition experiments. Two control peptides were also used. One consisted of 24 amino acid residues (SAAPSTPAAPSPORRPAETQQTQD) from a Babesia bovis 44-kDa variable merozoite surface antigen (15). The other consisted of 30 amino acid residues (SSSDSDEGEEITLPEDGVEEPDDVQIL) from a B. bovis 225-kDa spherical body protein (16). All peptides were dissolved in phosphate-buffered saline (pH 7.4) at a final concentration of 1 mg/ml. For peptide inhibition

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experiments, MAb 8WG16 was incubated with peptides (molar ratio, 1:200) at room temperature for 30 min before use in nuclear binding assays.

**Immunoblotting.** Immunoblotting was done as described elsewhere (27). Reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7 to 17% polyacrylamide) was used to separate proteins. Infected cell nuclei were analyzed to detect NA or RNA Pol II. Specific antibodies and controls used were as described for IFAs. Antibody binding was detected by incubation with horse radish peroxidase-conjugated secondary antibodies (goat anti-rabbit or -mouse) followed by development with an enhanced chemiluminescence kit (Amersham International plc., Buckinghamshire, England). Reactivity was localized by using Kodak XAR film. The molecular masses of proteins were estimated using Rainbow standards (14.3 to 220 kDa; Amersham).

**RESULTS**

**Binding of Pol II in infected cell nuclei by MAb 8WG16.** When nuclei isolated from infected cells were used in immunoblots, MAb 8WG16 bound specifically to proteins with molecular masses of approximately 240 and 210 kDa (Fig. 1). These masses are consistent with prominent hyper- and hypophosphorylated isoforms of the large subunit of Pol II. A nonspecific band at approximately 64 kDa was also observed.

MAb 8WG16 bound to nuclei from *T. spiralis*-infected muscle cells in a specific manner (Fig. 2A), with no evidence of nonspecific binding by the control antibody. The level of MAb 8WG16 binding to infected cell nuclei was significantly reduced by a heptapeptide repeat sequence from the C-terminal domain of Pol II (Fig. 2A, panels a, d, and e). Based on microfluorimetry measurements, inhibition was about 55%, with negligible inhibition by control peptides (Fig. 2B). When used in a Pol II enzyme-linked immunosorbent assay, the heptapeptide sequence caused about 50% inhibition of binding by this MAb (22). Collectively, the results indicated that infected cell nuclear proteins recognized by this MAb are Pol II. This conclusion is supported by the in situ distribution of antibody binding described below.

**Speckle distribution of Pol II in infected cell nuclei.** A generalized speckle distribution of Pol II in infected cell nuclei was observed (Fig. 2). Confocal microscopy better resolved this distribution (Fig. 3a). Localization of binding was nucleoplasmic, exclusive of nucleoli. When evaluated at levels separated by 1 μm within nuclei, distinct speckles could be observed. As in other reports, these speckles also showed interconnections (2). Virtually no diffuse component was found in the infected cell nuclei. MAb 8WG16 was previously shown to bind to both diffuse and speckle populations of Pol II in non-detergent-extracted nuclei (2). On extraction with nonionic detergents, the speckle population predominately remained. Since Nonidet P-40 is used for isolation of infected cell nuclei, the speckle distribution is expected for these nuclei. Therefore, the Pol II polypeptides being evaluated reside predominately in infected cell nuclear speckle domains. This speckle-localized population of Pol II is referred to as SL-Pol II.

**Colocalization of NA with SL-Pol II.** NA have a relatively diffuse nucleoplasmic distribution and colocalize with host chromatin (27). Dual-labeling experiments were done next to determine if the distributions of NA and SL-Pol II overlap. NA
occurred relatively homogeneously (but not completely) throughout the nucleoplasm, and the distribution of Pol II was contained completely within that of NA (Fig. 3b to d). Similar results were obtained when samples were analyzed at both 0.5- and 0.1-μm resolutions and at different levels within nuclei (data not shown). Therefore, we conclude that the distribution of NA is inclusive of the nuclear compartment(s) defined by SL-Pol II.

Enhanced Pol II levels in infected cell nuclei. To evaluate the relative levels of SL-Pol II in infected cell nuclei, comparisons were made with nuclei from infiltrating cells that are associated with infected cells in situ. These cells were chosen for several reasons. First, the two-dimensional protein profiles of infected cells are very similar to those of infiltrating cells but not differentiated skeletal muscle cells (12). Second, since no cell is known that has a phenotype matching that of *T. spiralis*-infected cells, infiltrating cells offer the best known basis for comparison. Infected cell nuclear preparations are routinely contaminated by infiltrating cell nuclei, a situation which prevents accurate protein quantitation for infected cell nuclei using whole nuclear lysates. However, these two populations can be visually separated by size, nuclear morphology, DNA content, and binding by anti-NA antibodies (11, 14).

In binding assays using MAb 8WG16, both the smaller size and the lower intensity of the fluorescence signal indicated lower Pol II levels in infiltrating versus infected cell nuclei (Fig. 4). From repeated comparisons by microfluorimetry, it was found that the mean fluorescence intensity of SL-Pol II in individual infected cell nuclei exceeded that in infiltrating cell nuclei by 3.9- to 6.8-fold (Table 1). Attempts to isolate differentiated muscle cell nuclei for comparisons have not been successful, since myofibrils do not dissolve under the conditions that we used to isolate other nuclei.

Next, regression analysis was done to assess a possible relationship between nuclear size and Pol II content. In experiment 2 (Table 1), measurements of Pol II content and nuclear diameter were obtained for infected cell nuclei and infiltrating cell nuclei. A low but significant correlation ($r^2 = 0.087, P < 0.001$) was observed between the Pol II content and the nuclear diameter for infected cell nuclei. When diameter measurements were converted to spherical volume, the results were approximately the same. Therefore, a portion of the increased size of infected cell nuclei could be related to Pol II content. In contrast, no significant relationship was observed between the Pol II content and the nuclear size for infiltrating cell nuclei ($P > 0.4$).

MBZ-mediated depletion of Pol II from infected cell nuclei. MBZ was used here to assess a possible parasite influence on host SL-Pol II levels. NA were depleted from infected cell nuclei by 4 days of MBZ treatment (Fig. 5A). After 4 days of treatment, minimal changes could be observed in the size and morphology of infected cell nuclei (26). By this time in the experiments done here, SL-Pol II levels were similar in infected cell nuclei from MBZ-treated and control mice (data not shown). By 10 days of treatment, alterations to infected cell

![FIG. 3. Pol II and NA distributions in infected cell nuclei. (a) Confocal microscopy of Pol II in speckle domains using MAb 8WG16 (2 μg ml$^{-1}$) in an IFA as described in the legend to Fig. 2. Green localizes MAb binding at one level, red localizes it at another level 1 μm away, and yellow shows the coincidence of binding at the two levels. (b to d) Dual labeling to determine the localizations of Pol II and NA in infected cell nuclei. (b) Pol II localization using MAb 8WG16. (c) NA localization using anti-NA antibody (α-DG p43) at the same level as that shown in panel b. (d) Merger of panels b and c. The yellow color shows colocalization. These results were obtained with 0.5-μm sections. Similar results were obtained with 0.1-μm sections and at different levels within nuclei. The results show that NA localize to speckle domains characterized by Pol II. Bar, 10 μm.

![FIG. 4. Comparison of Pol II in infected cell nuclei and inflammatory cell nuclei. The confocal picture shows infected cell nuclei (large arrows) and two infiltrating cell nuclei (small arrows). Nuclei were coisolated from chronically infected muscle cells and analyzed in an IFA using MAb 8WG16 as described in the legend to Fig. 2. Bar, 10 μm. Quantitation of immunofluorescence for the two classes of nuclei is shown in Table 1.](http://iai.asm.org/ on October 14, 2017 by guest)
nuclei were found to reach a plateau (26). After 10 days of MBZ treatment in the experiments done here, SL-Pol II levels were a mean of 70% higher in control than in MBZ-treated infected cell nuclei \((P, 0.001)\) (Fig. 5B). These results define an MBZ-sensitive SL-Pol II component in infected cell nuclei. In contrast, SL-Pol II levels in host infiltrating cells did not change significantly following MBZ treatment \((P, 0.4)\) (Fig. 5B). Hence, the effect on SL-Pol II was infected cell specific. Despite the MBZ-induced reduction in the level of infected cell nuclei, SL-Pol II remained at a significant level. We refer to this SL-Pol II component as the residual. This residual was found to remain elevated in infected cell nuclei by a mean of 120% compared to the level in infiltrating cells. In this regard, it may be relevant that infected cell nuclei have a mean DNA content that is estimated to range from 105 to 130% of that found in other host cells (11, 26). The possible relationship between DNA content and this residual is discussed below.

**DISCUSSION**

The results described here show that SL-Pol II occurred in infected cell nuclei at comparatively high levels. While diffusely distributed, NA colocalized to speckle domains defined by SL-Pol II, and the depletion of NA from infected cell nuclei was associated with a significant depletion of SL-Pol II from infected cell nuclei. Furthermore, following MBZ-induced depletion, the levels of SL-Pol II in the polyploid infected cell nuclei remained at approximately twice the SL-Pol II levels in the 2N nuclei of the infiltrating cells used for comparison. These results support the notion that *T. spiralis* infection leads to elevated SL-Pol II levels in infected cell nuclei. The results also address elements of the process leading to the expression of the infected cell phenotype. SL-Pol II levels were found to be significantly higher in infected cell nuclei than in nuclei from infiltrating cells. The cell best suited to make these comparisons remains debatable. However, since infiltrating cells have a two-dimensional protein profile that closely resembles that of infected cells (12), these cells offer the best known example for comparison. Both the magnitude of differences between infected cell nuclei and

**TABLE 1.** Comparison of Pol II levels in nuclei from *T. spiralis*-infected cells (NC) and associated infiltrating cells (IC), determined by IFA using MAb SWG16 and quantitated by microfluorimetry

<table>
<thead>
<tr>
<th>Expt</th>
<th>NC Mean (SD) relative fluorescence units</th>
<th>Sample size (n)</th>
<th>IC Mean (SD) relative fluorescence units</th>
<th>Sample size (n)</th>
<th>NC/IC ratio</th>
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<td>1</td>
<td>46.1 (24.6)</td>
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<td>11.8 (7.2)</td>
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<td>3.9</td>
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<td>6.8 (3.5)</td>
<td>50</td>
<td>6.8</td>
</tr>
<tr>
<td>3</td>
<td>64.2 (23.7)</td>
<td>47</td>
<td>12.4 (5.7)</td>
<td>36</td>
<td>5.2</td>
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*IFAs were carried out as described in the legend to Fig. 2. The nuclei were isolated from rats (experiments 1 and 2) or mice (experiment 3) harboring chronic *T. spiralis* infections. Relative fluorescence units obtained by microfluorimetry were used for comparisons. Differences between NC and IC were significant \((P < 0.001)\) in all comparisons.

**FIG. 5.** MBZ treatment of chronic *T. spiralis* infections depletes NA and Pol II from infected cell nuclei. (A) Immunoblot of infected cell nuclei \((n = 62,500)\) isolated from animals treated with vehicle alone (lane C) or MBZ (lane M) for 4 days. Proteins separated on 7.5 to 17.5% polyacrylamide gels were detected using anti-NA antibodies. The results demonstrate that NA were depleted from infected cell nuclei by the MBZ treatment. Size standards on the left are in kilodaltons. (B) Pol II levels in infected cell nuclei (NC) and infiltrating cell nuclei (IC) from mice treated as described for panel A. Measurements were made after 10 days of treatment. IFAs were conducted with MAb SWG16, and Pol II levels were quantitated by microfluorimetry. Error bars indicate standard deviations. The asterisk indicates significant \((P < 0.001)\) differences between the control and MBZ-treated infected cell nuclei. Results are representative of two experiments.
infiltrating cell nuclei (3.9- to 6.8-fold increase in the former) and the behavior of SL-Pol II in infected cell nuclei following MBZ treatment provide support for an elevation of SL-Pol II levels which is induced by the infection. In this regard, the measurement of Pol II levels in differentiated skeletal muscle cells is most desirable, but the isolation of nuclei from muscle tissue has not been possible.

Although quantitative variations in SL-Pol II levels among mammalian cells are not well documented, the relative abundance of SL-Pol II in infected cell nuclei seems remarkable. Transcription and transcript-processing enzymes have been colocalized in nuclear speckles (2, 7, 18, 25). Hence, the relative abundance of SL-Pol II in infected cell nuclei supports the suggestion that infected cell transcription is highly elevated. However, not all data link speckle domains with active transcription or processing (28). So, while intriguing by itself, the abundance of SL-Pol II also emphasizes the need to determine transcription rates in infected cell nuclei.

The enlarged size of infected cell nuclei and nucleoli was one stimulus to investigate Pol II levels. However, our analysis suggested only a minor influence of SL-Pol II levels on the size of infected cell nuclei. Since rRNAs constitute the majority of cellular RNA, this result may not be surprising. If the rate of transcription influences nuclear size, then Pol I-mediated transcription may provide the best correlate for nuclear size. Both the robust size of nucleoli and the diminution in the size of infected cell nuclei following MBZ treatment (26) support this possibility. However, the present study focused on Pol II levels, since NA provide the only recognized connection between the parasite and host nuclei, and NA are found exclusively in the host nucleoplasm. The results presented here indicate that measurements of Pol I protein and Pol I-mediated transcription levels in infected cell nuclei should be insightful.

A significant issue is the role that the infection (or parasite) plays in regulating the phenotype that is expressed by T. spiralis-infected muscle cells. The anthelmintic agent MBZ was used here and elsewhere (26) to assess the possible influence of the parasite on infected cell parameters. MBZ is expected to specifically affect the parasite and to inhibit parasite secretions. Consistent with this idea, MBZ treatment of infected mice causes the depletion of NA from infected cell nuclei (26; this study). Furthermore, MBZ-induced alterations in infected cells appear to be infected cell specific, since infiltrating cells have shown no parallel effects (26). This latter observation can now be extended to SL-Pol II levels. Finally, larval worms contained in MBZ-treated infected cells remain motile (26). Within the time constraints of our experiments, this evidence supports the notion that MBZ alters the infected cell phenotype by nullifying a parasite influence on the host cell without killing the parasite.

Based on the foregoing rationale, our results may reveal an active parasite influence on host SL-Pol II levels during chronic infection. This possibility stems from the 70% elevation of SL-Pol II in infected cell nuclei in control versus MBZ-treated mice. A similar effect was observed with the functionally dissimilar nuclear lamins A and C, where levels were 200% higher in control nuclei (26). These changes define an MBZ-sensitive component for the populations of each of these proteins. If the MBZ-sensitive component reflects the magnitude of influence that T. spiralis chronically exerts on host muscle cells, then the estimates for SL-Pol II and lamin A and C (means of 70 and 200%, respectively) are measures of that influence. While the possible secretion of parasite Pol II into the host cell cannot be excluded, MAb SWG16 was negative for binding to excretory-secretory products of muscle larvae, as assessed by immunoblotting (data not shown).

Despite the demonstration of an MBZ-sensitive component for host cell characteristics, residual levels of SL-Pol II (this study) and lamin A and C (26) persisted in infected cell nuclei following MBZ treatment. Here, we consider that this residual might reflect a permanent host cell change that is induced by the infection but requires no chronic parasite input. For example, this residual component was significantly higher in infected cell nuclei than in infiltrating cell nuclei. For SL-Pol II, the residual was a mean of 120% higher than the level in infiltrating cell nuclei. It may be relevant here that the mean DNA content of infected cell nuclei has been estimated to exceed that of infiltrating cell nuclei by 105 to 130% (11, 26). Hence, the residual SL-Pol II levels might be related to the DNA content of infected cell nuclei.

If residual SL-Pol II reflects the DNA content of infected cell nuclei, then it is likely to represent a permanent change induced by infection. For instance, DNA replication occurs in infected cell nuclei at between 4 and 6 dpi (11). The polyploid condition of these nuclei represents an early and apparently permanent change in our experimental system (11). Additionally, MBZ treatment did not cause a reduction in the ploidy of infected cell nuclei (26), suggesting that constant parasite input is not required to maintain this condition.

Based on the foregoing discussion, current data may be explained by a multistep process in which T. spiralis influences host SL-Pol II and other infected cell characteristics. One step is infection-induced amplification of the host genome, which might translate into a concomitant increase in basal levels of gene expression. Another step is the active release of parasite products that cause a chronic and additive elevation of host cell products. Since nuclear hypertrophy appears to precede DNA replication (4), the order of steps presented is not intended to reflect biological order. This general model could account, at least in part, for other exceptional characteristics of the infected cell. For instance, acid phosphatase activity and collagen gene expression both appear to be elevated to unusual levels (13, 20), and infected cell collagen is expected to contribute to the unique collagen capsule that envelopes infected muscle cells.

Finally, NA represent potential candidates for chronic regulators of infected cell characteristics. Here we show that NA colocalize with SL-Pol II, and NA depletion from infected cell nuclei was followed by depletion of SL-Pol II. These results introduce two new points. The first is that NA are located where interactions with host transcriptional proteins are possible. It is now important to determine if this localization is coincidental, due to a diffuse NA distribution, or functional. The second point is that, independent of such interactions, NA may contribute to the elevation of SL-Pol II levels.

Determining if similar kinds of host cell effects occur with Trichinella spp. which do not induce an infected cell capsule (e.g., T. pseudospiralis) remains problematic. Our methods relied on the capsule for the initial isolation of infected cells, from which nuclei were obtained. Hence, addressing related
questions about host cell nuclear interactions for nonencapsulating species will require alternative approaches. In this regard, the *T. spiralis* system has been useful for clarifying questions that might be investigated for nonencapsulating species.

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