Annexin XXI (ANX21) of Giardia lamblia Has Sequence Motifs Uniquely Shared by Giardial Annexins and Is Specifically Localized in the Flagella

We have identified a novel annexin, ANX21, in trophozoites of Giardia lamblia. The nucleotide sequence encoding this protein deviated from a published sequence in predicting an additional endonexin fold in the fourth annexin domain. In addition, several motifs exclusively shared by other annexins of G. lamblia in their predicted fourth repeat and predicted to be localized on the opposite (concave) surface of the molecule became apparent. Western blots of trophozoite fractions probed with antiseraum against the recombinant protein indicated that this annexin, like the other giardial annexins ANX19 and ANX20, associates with phospholipids in the presence of Ca\(^{2+}\). Finally, confocal laser scanning of trophozoites showed that the protein, apart from the median body, was exclusively localized in the eight flagella. Together, these data suggest that ANX21 may function as a Ca\(^{2+}\)-regulated structural element linking phospholipid bilayer and underlying axoneme.

Giardia lamblia (syn. Giardia intestinalis, Giardia duodenalis), a group of diplomonadid parasitic protists, is classified as early branching eukaryotes (1). G. lamblia occurs throughout the world and triggers a form of diarrhea called giardiasis (2). Its life cycle consists of two stages: the infective, immobile cyst form that by virtue of its tough cell wall is able to survive the inhospitable conditions of the host’s stomach, and the vegetative, mobile trophozoite form that attaches to the epithelial cells of the gut. It does so with the help of a cytoskeletal structure called ventral disk, which probably functions as a suction cup. The ventral disk consists of spiraling microtubuli with flat structural elements called microribbons protruding from them into the cytoplasm (3). The edges of these microribbons contain two proteins that were originally designated as α-giardins (4, 5) and shown to be associated with the cytoskeletal fraction by detergent extraction of the insoluble cell pellet (6). Based on their predicted amino acid sequence, they were later identified as members (ANX19 and ANX20) of the annexin (ANX) family (7). Annexins are eukaryotic proteins that usually bind to phospholipid bilayers in a Ca\(^{2+}\)-dependent manner (for an exception, see Ref. 8) and supposedly play a role in Ca\(^{2+}\)-dependent membrane dynamics (9). They consist of four homologous, mainly α-helical domains folded into a concave/convex shape. In annexins of higher eukaryotes, each of these four domains possesses the canonical repeat GXGTD followed by an aspartate or glutamate residue 38 positions downstream that forms a loop (AB loop) at the convex surface and functions as high affinity (“type II”) Ca\(^{2+}\)-binding site (10). Other Ca\(^{2+}\)-binding sites with lower affinity (“type III”) provided by spatially clustered carboxylate groups are likewise positioned at the convex surface of the molecule. Most annexins exhibit an ion channel activity with ion transport assumed to occur through a central pore lined by charged residues, particularly glutamate and arginine (9). Although the giardial annexins ANX19 (11) and ANX20, in addition to interacting with the cytoskeleton fraction (6, 12), bind to phospholipids in a Ca\(^{2+}\)-dependent manner, they lack an endonexin fold and do not exhibit ion channel activity.

In a report on the structure of a gene encoding a pyruvate-ferredoxin oxidoreductase from G. lamblia, a flanking complementary DNA sequence putatively encoding an additional annexin has been identified (13). To extend the open reading frame and to increase the overall similarity of the predicted ANX21 to other annexins, the authors postulated an insert of primary DNA sequence putatively encoding an additional annexin in predicting an additional endonexin fold in their fourth domain (14). We here present a corrected nucleotide sequence downstream that forms a loop (AB loop) at the convex surface and conserves giardin motifs on its concave surface. In trophozoites, ANX21 was exclusively localized in the flagella. These data are consistent with a model in which ANX21 functions as a Ca\(^{2+}\)-regulated structural element linking the flagellar membrane and the axoneme.

**EXPERIMENTAL PROCEDURES**

**Cells**—Trophozoites of G. lamblia strain WB-C6 (ATCC 30957) were cultured in Keister’s modified TYI-S-33 medium (15). Cells were harvested at the end of the logarithmic phase (after 3–4 days), washed three times in phosphate-buffered saline (PBS; 150 mM NaCl, 20 mM K\(_2\)HPO\(_4\); 7.5 mM KH\(_2\)PO\(_4\); pH 6.9), and stored at −20 °C in the presence of 10 μM (final concentration) trans-epoxysuccinyl-L-leucylamide-(4-guanidinobutane (E-64), a potent inhibitor of cysteine proteinases.

**Nucleic Acid Manipulations—Genomic DNA was isolated from fresh trophozoites using an Elu-Quick kit (Schleicher and Schuell). To amplify the open reading frame, a primer pair was designed complementary DNA sequence (14).**

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) L17221.

*This work was supported by the Deutsche Forschungsgemeinschaft by a grant (to A.S.) within the framework of the graduate college “Molecular Physiology.” The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

To whom correspondence should be addressed: Faculty of Biology/Chemistry, Barbarastrasse 11, D49069 Osnabrueck, Germany. Tel.: 49541-9692888; Fax: 49541-9692870; E-mail: scholze@biologie.uni-osnabrueck.de.

The abbreviations used are: ANX, annexin; PBS, phosphate-buffered saline; CAPS, 3-(cyclohexylamino)propanesulfonic acid; E-64, trans-epoxysuccinyl-L-leucylamide-(4-guanidinobutane.

---

2 M. Hauptmann, unpublished data.
open reading frame from position 318 to 1369 of the DNA sequence deposited in the sequence data base (GenBank™ L17221). PCR on genomic DNA as template was performed using Pfu-polymerase (Stratagene, Heidelberg). The PCR profile was as follows: 5 min at 94 °C, 45 cycles with 2 min at 55 °C, 2 min at 72 °C, and 1 min at 94 °C, then 2 min at 55 °C, and 5 min at 72 °C. The amplification product was purified using a QIAEx-II Gel-Extraction-Kit (Qiagen, Hilden Germany) according to the instructions of the manufacturer, subcloned into pBSK, and sequenced by the automated dyeoxy chain termination method (MWG Biotech and in house). The sequence was determined twice in both directions.

Expression of Recombinant Protein and Antibody Production—The amplification product was digested with BamHI and ligated in frame into the multiple cloning site of the expression vector pET16b, which contains a 5′-extension sequence coding for 10 histidine residues (His-tag). After transfection of the construct into Escherichia coli BL21 and induction with isopropyl-1-thio-galactoside, the overproduced protein product was partially present in the soluble fraction as checked by SDS-PAGE. For purification, the soluble E. coli extract was adjusted to 25 mM imidazole, the mixture applied onto an Ni-NTA column, and the recombinant protein eluted with 20 mM Tris-HCl, pH 7.9, containing 250 mM imidazole. This yielded a >95% pure recombinant protein judging from the SDS-PAGE (data not shown), which was sent out for the immunization of rabbits (Eurogentec, Belgium). On a Western blot, the extract of E. coli containing the recombinant protein and of the purified recombinant protein, the antiserum (1:1000) reacted with a single protein band at an apparent M, 40,500; the same band was detected with antipenta-His antiserum (Qiagen, 1:2000; data not shown).

Isolation of Annexins and Binding Studies—Annexins were isolated from crude trophozoite homogenates by EGTA extraction according to Ref. 16. Pellet and supernatant fractions were analyzed by SDS-PAGE and Western blotting. For phospholipid binding, 400 µL of 20 mM Hepes buffer was brought to pH 7.4 with NaOH containing 100 mM KCl, 2 mM MgCl₂, 1 mM EGTA, and 0.5 mg multilamellar liposomes (brain extract; Sigma) was added to 100 µL of soluble EGTA extract containing 30 µg of protein. The mixture was incubated for 40 min at room temperature under shaking and then centrifuged for 10 min at 15,000 × g. In a parallel experiment, the free Ca²⁺–concentration in the mixture was adjusted to 1 mM, and in control incubations the brain extract was omitted. For isolation of detergent-insoluble cytoskeletal proteins cells were homogenized in the presence of 4 mM Ca²⁺, the homogenate were centrifuged for 10 min at 15,000 × g, and the pellet fraction subsequently was extracted with 0.5% (w/v) Triton X-100. The extract was mixed in equal volume of ice-cold 10% (w/v) trichloroacetic acid, and equivalent fractions of the precipitated protein (Triton X-100 extract) and the Triton-insoluble pellet were analyzed by SDS-PAGE and Western blotting.

Isolation of Flagella—Trophozoite flagella were isolated by the method of Clark and Holberton (17). Briefly, the cells were washed twice by centrifugation in 0.25 M sucrose and resuspended in TMSK buffer consisting of 30 mM Tris, 2.5 mM MgSO₄, 0.2 M sucrose, 25 mM KCl, 1 mM EDTA, and 0.005% (w/v) phenylmethylsulfonyl fluoride, pH 7.4. The suspension was homogenized for 1 min with an Ultra-Turrax (Braun Melsungen, Germany). Cell bodies were removed by centrifugation for 14 to 19% and to that of human ANX5 (22) from 11 to 19% and reveals the presence of Annexin (20) in a CAPS/NaOH-buffer, pH 11.0, and equivalent fractions of the precipitated protein (Triton X-100 extract) and the Triton-insoluble pellet were analyzed by SDS-PAGE and Western blotting.

Isolation of Flagella—Trophozoite flagella were isolated by the method of Clark and Holberton (17). Briefly, the cells were washed twice by centrifugation in 0.25 M sucrose and resuspended in TMSK buffer consisting of 30 mM Tris, 2.5 mM MgSO₄, 0.2 M sucrose, 25 mM KCl, 1 mM EDTA, and 0.005% (w/v) phenylmethylsulfonyl fluoride, pH 7.4. The suspension was homogenized for 1 min with an Ultra-Turrax (Braun Melsungen, Germany). Cell bodies were removed by centrifugation for 14 to 19% and to that of human ANX5 (22) from 11 to 19% and reveals the presence of Annexin (20) in a CAPS/NaOH-buffer, pH 11.0, and equivalent fractions of the precipitated protein (Triton X-100 extract) and the Triton-insoluble pellet were analyzed by SDS-PAGE and Western blotting.

Nucleotide Sequence of anx21 and Its Implications for the Predicted Protein—To check for the postulated insertion of an undefined nucleotide (14) in the published sequence of anx21 (13) and to pinpoint its nature and exact position we amplified the complete open reading frame using genomic DNA as template and sequenced the amplified product (sequence available from the EMBL data base under the accession number AJ271737). While confirming an insertion of one base (identified as G), we found its exact position to occur at nucleotide number 9836 rather than 9846, as proposed by Morgan and Fernandez (14), of the complementary sequence. This correction of the published nucleotide sequence increases the identity of the second half of the sequence to that of giardial ANX19–20 from 14 to 19% and to that of human ANX5 (22) from 11 to 19% and reveals an additionalendonin fold (GSGSD/S8/E) at positions 257–261 and 299 (Fig. 1; numbering adapted to ANX5 sequence). The corrected predicted C-terminal sequence now shares several sequence motifs with ANX19 and ANX20, namely IT/GA/M at 268–270 and 272, KXXY (X stands for a variable residue) at 281–285, DXER at 293–296 and Trp at 311 (Fig. 1). (Some of these residues have been boxed as “prost- specific” in Ref. 13, Fig. 6). Meanwhile, the genome data base of G. lamblia (23) has yielded >13 other independent sequences encoding putative annexins, and strikingly, one of these are predicted to exhibit the same sequence motifs corresponding to ANX19–21 but not the endonoxin fold shared by ANX21 and ANX5; data not shown), whereas these motifs fail in all known annexins from other organisms. In most of these sequences a positively charged residue (usually Arg) follows the tryptophan residue at 311. In a molecular model the indole ring of this tryptophan together with the KXXY motif ends up on the concave surface of the molecule, opposite the endonoxin

RESULTS AND DISCUSSION

Nucleotide Sequence of anx21 and Its Implications for the Predicted Protein—To check for the postulated insertion of an undefined nucleotide (14) in the published sequence of anx21 (13) and to pinpoint its nature and exact position we amplified the complete open reading frame using genomic DNA as template and sequenced the amplified product (sequence available from the EMBL data base under the accession number AJ271737). While confirming an insertion of one base (identified as G), we found its exact position to occur at nucleotide number 9836 rather than 9846, as proposed by Morgan and Fernandez (14), of the complementary sequence. This correction of the published nucleotide sequence increases the identity of the second half of the sequence to that of giardial ANX19–20 from 14 to 19% and to that of human ANX5 (22) from 11 to 19% and reveals an additionalendonin fold (GSGSD/S8/E) at positions 257–261 and 299 (Fig. 1; numbering adapted to ANX5 sequence). The corrected predicted C-terminal sequence now shares several sequence motifs with ANX19 and ANX20, namely IT/GA/M at 268–270 and 272, KXXY (X stands for a variable residue) at 281–285, DXER at 293–296 and Trp at 311 (Fig. 1). (Some of these residues have been boxed as “prost-specific” in Ref. 13, Fig. 6). Meanwhile, the genome data base of G. lamblia (23) has yielded >13 other independent sequences encoding putative annexins, and strikingly, one of these are predicted to exhibit the same sequence motifs corresponding to ANX19–21 but not the endonoxin fold shared by ANX21 and ANX5; data not shown), whereas these motifs fail in all known annexins from other organisms. In most of these sequences a positively charged residue (usually Arg) follows the tryptophan residue at 311. In a molecular model the indole ring of this tryptophan together with the KXXY motif ends up on the concave surface of the molecule, opposite the endonoxin...
fold (Fig. 2), suggesting that these residues could constitute a binding site for a cytoplasmic interaction partner.

Expression of anx21 in Trophozoites and in E. coli—Both Southern blot analysis and data base searching indicated that the G. lamblia genome possesses just one nucleotide sequence encoding ANX21 (data not shown). Northern blot analysis confirmed expression of this anx21 gene in trophozoites with a transcript size (~1050 nt) that leaves ~40 nt for the 5' and 3'-untranslated regions (Fig. 3). To raise anti-ANX21 antibodies, we overproduced the recombinant, His-tagged protein heterologously in E. coli (for details, see "Experimental Procedures"). Probing of Western blots with the antiserum raised against the recombinant protein confirmed that ANX21 is present in trophozoites (Fig. 4).

Association of ANX21 with Phospholipids and with the Detergent-insoluble Cytoskeletal Fraction—To investigate the association of ANX21 with negatively charged phospholipids, we extracted trophozoite homogenate with EGTA and incubated the soluble supernatant with multilamellar liposomes prepared from brain extract. Fig. 4A shows that the extracted ANX21, just like ANX19 (11) and ANX20 (data not shown), ended up in the pellet fraction in the presence of excess Ca2+ (lane 4) but remained in the supernatant in its absence (lane 1).

This effect was strictly dependent on the presence of the liposomes (lanes 5–6). These data confirm that ANX21 behaves as a classical annexin in associating with negatively charged phospholipids in a Ca2+-dependent way. In a complementary set of experiments, we homogenized the cells in the presence of 4 mM free Ca2+ and subjected the homogenate to centrifugation, which should be dissolved into floating micellar structures by the detergent; we interpret these data to mean that ANX21, apart from binding to phospholipids, also associates with detergent-insoluble cytoskeletal elements in the presence of Ca2+. As expected, in the presence of endogenous phospholipids, ANX21 remained in the insoluble fraction (lane 5; Fig. 4B). Likewise, when the pellet fraction was subsequently extracted with detergent, ANX21 remained in the pellet fraction (Fig. 4B, lane 4). However, when this pellet was treated with an 1 mM excess of EGTA, ANX21 went into solution (lane 5, Fig. 4B). As any phospholipid membranes that had been precipitated from the homogenate should be dissolved into floating micellar structures by the detergent, we interpret these data as evidence that ANX21, apart from binding to phospholipids, also associates with detergent-insoluble cytoskeletal elements in the presence of Ca2+. An association with the detergent-extracted cytoskeletal fraction has also been reported (17) for ANX19 and ANX20 (at that time denoted as α-giardins). Combining the model of Fig. 2 with the fractionation behavior

![Molecular model of ANX21](image1.png)

**Fig. 2. Molecular model of ANX21.** The model was computed by the SWISS-MODEL program (31) using the coordinates of related annexins and visualized with the WebLabViewer. The fourth domain is marked in dark gray. G. lamblia-specific residues on the concave side of the molecule are labeled. The fourth-domain endonexin fold comprises an extended loop on the convex side of the molecule.

![Northern analysis of anx21 expression](image2.png)

**Fig. 3. Northern analysis of anx21 expression.** For Northern blot analysis, 8 µg of trophozoite poly(A)+ RNA (isolated with superparamagnetic oligo(dT)-covered beads; Dynal) was electrophoresed, blotted, and hybridized at 60 °C with a digoxigenin-labeled amplification product encoding ANX21. The blot was washed at 60 °C in 2× SSC and 1× SSC for 15 min each. The hybridizing fragment was visualized by chemiluminescence. The positions of size markers (nt) are indicated at the left of the blot.

![Phospholipid binding of trophozoite ANX21 and its association with the detergent-insoluble cytoskeletal fraction](image3.png)

**Fig. 4. Phospholipid binding of trophozoite ANX21 and its association with the detergent-insoluble cytoskeletal fraction.** A, phospholipid binding of ANX21 obtained by EGTA extraction of crude trophozoite homogenate. Multilamellar liposomes prepared from brain extract phospholipids were added in the absence (lanes 1–2) or presence (lanes 3–4) of 1 mM free Ca2+; the supernatant (s) and pellet (p) fractions were subjected to SDS-PAGE, blotted, and probed with anti-ANX21 antiserum. Lanes 5–6 show control without phospholipids. B, association of ANX21 with the detergent-insoluble fraction. A crude trophozoite homogenate prepared in the presence of 1 mM free Ca2+ either without detergent (lanes 1–2) or after subsequent extraction with Triton X-100 (lanes 3–4) was fractionated into supernatant (s) and pellet (p) fractions and probed as above. Lane 5 shows EGTA extract of pellet from lane 4. Each lane contained 30 µg of protein. For further details, see "Experimental Procedures."

![Immunolocalization of ANX21](image4.png)

**Fig. 5. Immunolocalization of ANX21.** A, fixed and permeabilized trophozoites incubated with antiserum against ANX21. B, fixed and permeabilized trophozoites incubated with an antibody against tubulin. Bar length, 5 µm. C, Western blot of purified flagella probed with anti-tubulin and anti-ANX21 antiserum. Tub, tubulin. For further details, see "Experimental Procedures."

---

Downloaded from http://www.jbc.org/ by guest on July 18, 2018
documented in Fig. 4, we speculate that the *G. lamblia*-specific motifs of ANX19–21 are responsible for interaction (directly or indirectly) with detergent-insoluble cytoskeletal elements.

**Immunolocalization of ANX21 in Trophozoites**—In fixed and permeabilized trophozoites, ANX21 was exclusively localized in the eight flagella (and in those cells where it was observable, in the median body) (Fig. 5A). Control incubations with antisera against tubulin (Fig. 5B) and a proteasome subunit (not shown) indicated that this restricted distribution was not due to a lack of permeability of the cells. In agreement with these data, Western blot analysis of isolated flagella revealed cross-reaction with both the tubulin and ANX21 antibodies (Fig. 5C) but not with anti-ANX19 antibodies (data not shown). Together with our observation that ANX21 interacts with both phospholipids and the detergent-insoluble cytoskeletal fraction in a Ca$^{2+}$-dependent way, we interpret the flagellar localization to mean that ANX21 may play a Ca$^{2+}$-regulated structural role in trophozoite motility. Specifically, our data would fit in with a model in which binding of Ca$^{2+}$ to the convex surface of ANX21, apart from increasing the affinity of this surface to the flagellar membrane, induces a conformational change at the concave surface that enables the latter to interact with a cytoskeletal element of the axoneme (possibly an adapter protein). In the literature, there are precedents for both Ca$^{2+}$-dependent binding of annexins to cytoskeletal proteins and for the localization of annexins in flagella or cilia. Thus, many vertebrate annexins bind F-actin in a Ca$^{2+}$-dependent way (24). For ANX2, this binding has been shown to depend upon the nine C-terminal motifs conserved in the last domains of giardial annexins (Fig. 21) may be important; consequently, our future experiments will be directed at identifying the corresponding protein binding partner(s) of these annexins.

**Acknowledgments**—We thank Bettina Flockenhaus for expert technical advice and Dr. Tilly Bakker-Grunwald for constructive criticism of the manuscript.

**REFERENCES**

1. Roger, A. J. (1999) *Amer. Nat.* **154**, 146–163
2. Adam, R. D. (2001) *Clin. Microbiol. Rev.* **14**, 447–475
3. Kulda, J., and Nohynekova, E. (1995) in *Parasite Protozoa* (Kreier, J. P., ed), 2nd Ed., Vol. 10, pp. 225–422, Academic Press, NY
4. Peattie, D. A., Alonso, R. A., Hein, A., and Caulfield, J. P. (1989) *J. Cell Biol.* **109**, 2325–2335
5. Alonso, R. A., and Peattie, D. A. (1992) *Mol. Biochem. Parasitol.* **50**, 95–104
6. Crossley, R., and Holberton, D. (1985) *J. Cell Sci.* **70**, 205–231
7. Morgan, O. R., and Fernandez, M.-P. (1995) *Mol. Biol. Ecol.* **12**, 967–979
8. Jost, M., Zerinscher, D., Seemann, J., Weber, K., and Gerke, V. (1997) *J. Cell. Sci.* **110**, 221–228
9. Gerke, V., and Moa, E. S. (1997) *Biochim. Biophys. Acta* **1357**, 129–154
10. Seaton, B. A. (1996) *Annu. Rev. Biochem.* **65**, 15–29, Springer-Verlag New York Inc., New York
11. Bauer, B., Engelbrecht, S., Bakker-Grunwald, T., and Scholze, H. (1999) *FEBS Microbiol. Lett.* **173**, 147–153
12. Peattie, D. A. (1990) *Parasitol. Today* **6**, 52–56
13. Townsend, S. M., Urcopp, J. A., and Urcopp, P. (1996) *Mol. Biochem. Parasitol.* **79**, 183–193
14. Morgan, O. R., and Pillar Fernandez, M. (1997) *J. Mol. Evol.* **44**, 178–188
15. Keister, D. B. (1983) *Trans. R. Soc. Trop. Med. Hyg.* **77**, 487–488
16. Boustead, C. M., Walker, J. H., and Geisow, M. J. (1988) *FEBS Lett.* **233**, 233–238
17. Clark, J. T., and Holberton, D. V. (1988) *Parasitol. Res.* **74**, 415–423
18. Douglas, M., Finkelstein, D., and Butow, R. A. (1979) *Methods Enzymol.* **56**, 58–66
19. Neuhoff, V., Arnold, N., Taube, D., and Ehrhardt, W. (1988) *Electrophoresis* **8**, 255–262
20. Matsudaira, P (1987) *J. Biol. Chem.* **262**, 10035–10038
21. Nohynekova, E., Draber, P., Reischig, J., and Kulda, J. (2000) *Eur. J. Cell Biol.* **79**, 438–445
22. Grundmann, U., Abel, K.-J., Bohn, H., Loebermann, H., Lottspeich, F., and Kuepper, H. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85**, 3708–3712
23. Mairhofer, A. G., Morrison, H. G., Nixon, J. E., Psammamneke, N. Q., Kim, U., Hinke, G., Crocker, M. K., Holder, M. E., Farr, R., Reich, C. I., Olsen, G. E., Aley, S. B., Adam, R. D., Gillin, F. D., and Sogin, M. L. (2000) *FEBS Microbiol. Lett.* **189**, 271–273
24. Khanna, N. C., Helwig, E. D., Kebuchi, N. W., Fitzpatrick, S., Bajwa, R., and Waismann, D. M. (1990) *Biochemistry* **29**, 4852–4862
25. Filipeenkov, N. R., and Waismann, D. M. (2001) *J. Biol. Chem.* **276**, 5310–5315
26. Mairhofer, A. G., Traverso, V., Marauz, S., and Massey-Harroche, D. (1996) *Ana. J. Physiol.* **270**, 863–871
27. Feinberg, J. M., Rainette, D. P., Kaelz, M., Dacheux, J. L., Dedman, J. R., and Weinman, S. J. (1991) *J. Histochem. Cytochem.* **39**, 855–963
28. Wu, Y., Nagahatani, N. G., and Ruben, L. (1992) *Biochem. J.* **287**, 187–193
29. Ridgley, E., Webster, P., Patton, C., and Ruben, L. (2000) *Mol. Biochem. Parasitol.* **109**, 195–201
30. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) *Nucleic Acids Res.* **22**, 4673–4680
31. Gues, N., and Peitsch, M. C. (1997) *Electrophoresis* **18**, 2714–2723
Annexin XXI (ANX21) of *Giardia lamblia* Has Sequence Motifs Uniquely Shared by Giardial Annexins and Is Specifically Localized in the Flagella

Anna Szkodowska, Monika C. M. Müller, Christoph Linke and Henning Scholze

*J. Biol. Chem. 2002, 277:25703-25706. doi: 10.1074/jbc.M203260200 originally published online May 2, 2002*

Access the most updated version of this article at doi: 10.1074/jbc.M203260200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 30 references, 8 of which can be accessed free at http://www.jbc.org/content/277/28/25703.full.html#ref-list-1