

Modeling Household Transmission of American Trypanosomiasis

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American trypanosomiasis, or Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi* and transmitted by blood-feeding triatomine bugs, is a chronic, frequently fatal infection that is common in Latin America. Neither adequate drugs nor a vaccine is available. A mathematical model calibrated to detailed household data from three villages in northwest Argentina shows that householders could greatly reduce the risk of human infection by excluding domestic animals, especially infected dogs, from bedrooms; removing potential refuges for bugs from walls and ceilings; and using domestically applied insecticides. Low-cost, locally practicable environmental management combined with intermittent use of insecticides can sustainably control transmission of *T. cruzi* to humans in rural Argentina and probably elsewhere.

Chagas disease, or American trypanosomiasis, is endemic in Central and South America. An estimated 16 to 18 million persons are infected with *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae), the causative agent of Chagas disease, and 100 million people (roughly one-quarter of the population) are at risk of infection (1). Despite decreasing rates of prevalence and incidence of *T. cruzi* infection (2, 3), Chagas disease remains a serious obstacle to health and economic development in Latin America, especially for the rural poor.

The repertoire of control measures is limited. Two drugs are curative in the acute and early chronic phase of infection but have adverse effects and may not always eliminate *T. cruzi*. No vaccines are available to prevent infection. Transmission may be interrupted by residual spraying to kill blood-feeding triatomine bugs (the vector of *T. cruzi*), screening blood donors, and treating infected infants born to infected mothers. A more controversial strategy for interrupting transmission is to divert bugs from humans by the use of animals that are not susceptible to *T. cruzi* (4). This strategy, called zooprophylaxis, is controversial for other vector-borne diseases (5) as well and may remain so for *T. cruzi* because it cannot be tested experimentally. Ethical considerations bar a randomized prospective field study in which the human prevalence of *T. cruzi* infection is compared between households that do, and those that do not, keep domestic animals in bedroom areas in the presence of domestic triatomine infestations.

Mathematical models of the transmission of *T. cruzi* infection are the next best available tool to understand the effects of alternative control strategies (6–14) [Web supplement gives further references (15)]. Here we present a model of the transmission of *T. cruzi* infection within an individual household. The model represents three vertebrate populations (humans, dogs, and chickens), the bug population, the parasite, and seasonality. Although many existing models of the transmission of infectious diseases use differential equations to represent changes in the prevalence of infection and in the population sizes of hosts and vectors, which are usually assumed to be large, the discrete formalism of the present model makes it easy to represent the age structure of one household's human population, the small numbers of domestic vertebrates, and the seasonal differences in host composition and exposures to bugs.

This model was developed in close connection with household data collected to support modeling in three rural villages, Amamá, Trinidad, and Mercedes, in the province of Santiago del Estero, northwest Argentina. The villages are situated within 9 km of each other in a semi-arid hardwood thorny forest habitat. In 65 houses of Amamá and Trinidad, the median household in 1993 had five people, about three infected dogs, no more than one cat, and 8 to 27 chickens and ducks (16).

In Argentina, transmission of *T. cruzi* to humans is minimal in fall and winter (April to August). Below 16° to 18°C, bugs cease development and feeding (17). In early spring, people sleep indoors, chickens are maximally present in bedroom areas (18), bugs are increasingly active and feeding, and the domiciliary bug population grows rapidly. In summer, chickens mainly roost outdoors, people usually move their raised beds outdoors to sleep on verandas or patios in front of their

bedrooms, and the size of the domiciliary bug population is maximal.

Domestic triatomine bugs take blood meals from household vertebrates to be able to develop from each instar to the next and to lay eggs. Keeping chickens in bedrooms in spring to protect them or their eggs from predation or theft increases the domestic bug population size (18, 19), most notably in the following summer. Because chickens cannot be infected with *T. cruzi*, the more often a domestic bug feeds on a chicken, the less likely the bug is to become infected with *T. cruzi*. Therefore, keeping chickens in bedrooms could decrease the prevalence rate of *T. cruzi* in bugs. In contrast, keeping infected dogs in the household increases both the bug population size (20) and bug prevalence of *T. cruzi* (21, 22). The summer population of large and late-stage *T. infestans* bugs, increased as a result of spring feeding on chickens, shifts feeding from chickens to humans or infected dogs in the hot summer season (16) when chickens are largely absent. The presence of chickens in bedroom areas decreased the prevalence of *T. cruzi* in bugs but increased the density of *T. cruzi*-infected bugs (23).

Mathematical modeling is required to understand the implications of these findings for the human prevalence of *T. cruzi* infection (4). We model only the transmission season, spring (September to mid-December) and summer (mid-December to March). The model represents the population of the parasite *T. cruzi* implicitly through prevalence rates. The human (or dog) prevalence rate gives the proportion of humans (or dogs) who are infected with *T. cruzi* (as measured by seropositivity) but does not distinguish among different phases of infection. In these calculations, all dogs are assumed to be infected, as field data show. The bug prevalence rates (in spring and in summer) give the fraction of bugs in each season that are infected with *T. cruzi*. In the model and real life, once a person, dog, or bug becomes infected, that individual remains infected for life (unless the person or dog is treated promptly; there are no treatments for bugs).

The model represents explicitly four populations: humans, dogs, chickens, and bugs. Humans are represented by the numbers of individuals in 5-year age groups: under age 5, ages 5 to 9, ages 10 to 14, etc. Dogs represent all mammalian domiciliary animals that are susceptible to infection with *T. cruzi*, provide blood meals to *T. infestans*, and are more attractive or accessible as a source of blood meals than humans. Chickens represent all avian domiciliary animals, which are not susceptible to infection with *T. cruzi* but which may provide blood meals to *T. infestans* and are more attractive or accessible blood sources than humans. In the model and henceforth here, "bugs" means exclusively fourth- and fifth-instar nymphs and adults. These stages include almost

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all bugs that are infected (23, 24) and capable of transmitting infection, and timed manual collections of bugs yield samples biased toward these stages.

Bugs are several times more likely to take their blood meals from dogs and chickens than from humans (20). For example, the ratio of dog blood meals to human blood meals in the engorged guts of bugs was 2.3 to 2.6 times the ratio of the number of dogs to the number of humans in a household in the spring-summer period. Similarly, bugs selected chickens for blood meals nearly five times as often as the number of chickens relative to humans. These ratios did not vary significantly among households with differing total numbers of humans, dogs, and chickens (20). Dogs and chickens are probably less attentive than humans in defending themselves against feedings by the bugs. Also dogs and chickens sleep or nest in bedroom places that are more accessible to the bugs than the raised beds on which people typically sleep, whether indoors or outdoors. We approximate the ratio of feedings on dogs and chickens relative to humans as 3, as a rough midpoint of the large range of variation of empirical estimates. The probability of infecting an initially uninfected bug in one full blood meal from an infected dog was 12 times that probability from infected children and 200 times that from infected adults (16).

The assumptions, variables, and formal structure of the model are detailed in the Web supplement (15). The model predicts how the numbers of humans, chickens, and infected dogs in a household and the physical-chemical conditions affect the prevalence of *T. cruzi* infection in humans and bugs, the number of infected and uninfected bugs, and the distribution of feeding contacts, by season. Only one parameter of the model was varied freely to improve the quantitative fit between observation and prediction: the probability of transmission of infection from an infected bug to an uninfected person ($t_{B \rightarrow H} = 0.0008$). This value is close to the 0.0009 estimated from one field study (9). For obvious ethical reasons, this probability cannot be estimated experimentally and is largely unknown.

Given the complexity of the natural system and the relative simplicity of any mathematical model, qualitative agreement is the most that can be hoped for from the comparisons of model predictions and field observations. The following comparisons suggest that the qualitative predictions of the model give some insights into the real household transmission of *T. cruzi*.

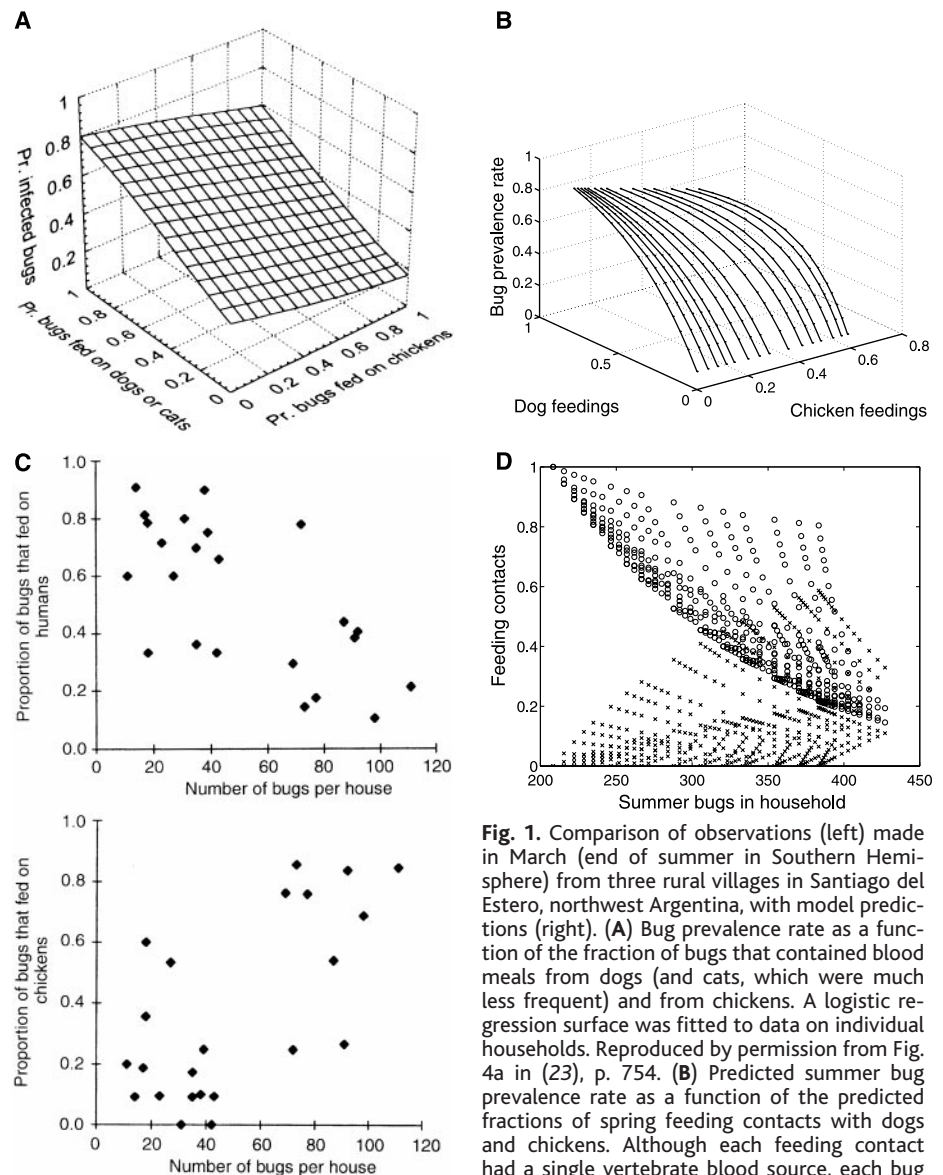
Empirically (Fig. 1A) and theoretically (Fig. 1B), the bug prevalence rate increased rapidly with the fraction of bugs that took blood meals from infected dogs (or cats, which were relatively rare) when the fraction of bugs that took blood meals from chickens stayed constant. The bug prevalence rate de-

creased slowly with an increasing fraction of bugs that took blood meals from chickens when the fraction of bugs that took blood meals from infected dogs stayed constant.

Empirically (Fig. 1C) and theoretically (Fig. 1D), as the relative density of bugs collected per unit of search effort at the end of summer increased, the fraction of bugs that contained blood meals from humans decreased while the observed fraction of bugs that contained blood meals from chickens increased. The model predictions are shown for spring and summer combined because blood meals from both seasons were probably detectable in bugs collected at the end of summer (16). The source of a full

blood meal taken at an earlier instar is detectable for up to 3 months from blood taken from the gut of a later instar of the same individual bug. The intuitive explanation as to why both field data and the model show that increasing number of summer bugs are associated with fewer human feeding contacts and more chicken feeding contacts is that, for any given number of dogs, the summer bug population increases as a result of increasing availability of chickens in spring, and chickens are more accessible or attractive than humans as blood meal sources for the bugs.

The two principal predictions of the model are as follows:



one species of vertebrate. A given bug may be counted more than once in (A), but each feeding contact counts only once in (B). (C) Fraction of bugs that contained blood meals from humans (top) and chickens (bottom) as a function of the bug density per unit of searching effort. Reproduced by permission from Fig. 2, a and b, in (20), p. 706. Each point belongs to a different household. (D) Predicted fractions of feeding contacts on humans (o) and chickens (x) in spring and summer (combined because of the carryover of blood meals) as a function of the predicted number of summer bugs for all combinations of numbers of dogs and chickens considered.

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1) The worst thing householders can do, from the point of view of limiting *T. cruzi* prevalence, is to keep roughly two infected domestic dogs (Fig. 2I). This is precisely what many households do. The intuitive explanation why the model predicts that roughly two infected dogs should be pessimal for human prevalence is that each dog is as attractive as three humans, and five humans are assumed in the household, so two dogs is roughly equivalent to the five humans from the bugs' point of view. As the number of infected dogs increases up to two, the infected dogs are an increasing and highly infective source of *T. cruzi*. As the number of infected dogs increases beyond two, the additional infected dogs contribute infection to many already infected bugs but also divert proportionally more of the feeding bugs from humans. This intuitive account explains not only the location of the peak human prevalence rate around two infected dogs, but also explains why the rise of human prevalence from zero to two infected dogs is faster than the very gradual fall of human prevalence as the number of infected dogs increases beyond two.

2) Elimination of infected dogs from a household with infected people is nearly sufficient to extinguish transmission of *T. cruzi*, barring reintroduction of infected dogs, children, or bugs (Fig. 2, E, F, G, and I).

The model makes many additional predictions (Fig. 2).

3.1) The number of spring bugs increases

with the number of dogs but is independent of the number of chickens (Fig. 2A). Chickens, kept in the household in spring, have no immediate effect on spring bugs because the model assumes a lag of one season between the availability of blood sources and the recruitment of late-instar and adult bugs.

3.2) The number of summer bugs increases with both dogs and chickens in the previous spring (Fig. 2B), as 1988–1989 data at Amamá, Trinidad, and Mercedes showed (18–20).

3.3) The fraction of spring bugs' feeding contacts with humans (Fig. 2C) decreases symmetrically, rapidly at first and then more slowly, with more dogs and chickens.

3.4) The fraction of spring bugs' feeding contacts with infected dogs (Fig. 2D) increases at a diminishing rate with more infected dogs and decreases with more chickens. The fraction of spring bugs' feeding contacts with chickens (not shown) is obtained by exchanging the axes of Fig. 2D for chickens and dogs.

3.5) The fraction of spring bugs infected with *T. cruzi* (the spring bug prevalence rate) increases at a diminishing rate with more infected dogs and decreases slightly with more chickens (Fig. 2E). Even when there are no infected dogs and no chickens, the spring bug prevalence rate remains positive because infected people remain in the household (by assumption in the model, and because of the persistent presence of chronically infected

adults in the field). When there are no dogs, an increase in the number of chickens diverts bugs from feeding on humans to feeding on unsusceptible chickens and gradually reduces the bug prevalence rate.

3.6) The prevalence rate in summer bugs (not shown) increases with the number of infected dogs as in Fig. 2E. Observed bug prevalence rates of *T. cruzi* infection increased with the number of infected dogs in 1988–1989 in Amamá (21). In Trinidad and Mercedes, the mean bug prevalence rates were 16, 41, 68, and 47% in households with zero, one, two, and three infected dogs, respectively (22). The positive mean bug prevalence rate in households with zero infected dogs is due to the presence of infected cats. Bug prevalence rates were 4% in households with no infected dogs or cats and 35% in households with an infected cat and no infected dog.

3.7) The spring number of infected bugs (Fig. 2F) parallels the spring bug prevalence rate (Fig. 2E) because the spring bug population size is independent of the number of chickens (Fig. 2A).

3.8) For any fixed number of dogs, households with more chickens have slightly more infected summer bugs (Fig. 2G) and substantially more total summer bugs (Fig. 2B). Thus, the presence of chickens in bedrooms in spring decreases the number of infected bugs in spring and increases the number of

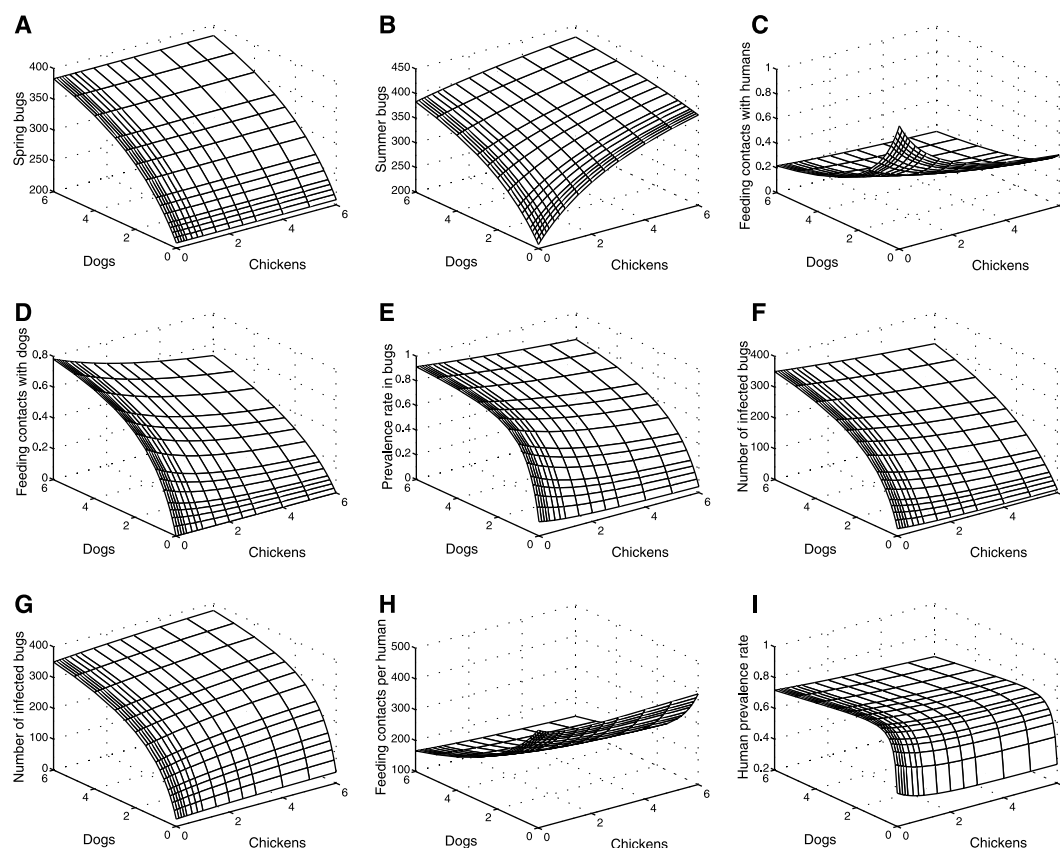


Fig. 2. Predictions from a model of the household transmission of *T. cruzi* for selected combinations of chickens and infected dogs. (A) Number of bugs in household in spring. (B) Number of bugs in household in summer. (C) Fraction of feeding contacts from humans in bugs captured in spring. (D) Fraction of feeding contacts from dogs in bugs captured in spring. (E) Prevalence rate in bugs captured in spring. (F) Number of infected spring bugs. (G) Number of infected summer bugs. (H) Number of feeding contacts per human per year. (I) Human prevalence rate, or fraction of humans infected, at steady state.

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infected bugs in summer. The summer increase in infected bugs is slightly smaller than the absolute spring decrease in infected bugs for any positive number of chickens in spring.

3.9) The number of feeding contacts per human per year (Fig. 2H) decreases rapidly with increasing numbers of dogs and decreases very slightly with increasing numbers of chickens, because the chickens divert feeding contacts only in spring.

3.10) The number of potentially infective feeding contacts per human per year is the product of the number of feeding contacts per human per year (Fig. 2H) times a weighted average of the spring (Fig. 2E) and summer bug prevalence rates. The number of potentially infective feeding contacts (not shown, but similar in shape to Fig. 2I) rises to a peak with an increase from 0 dogs to 1.5 infected dogs, and then begins to decline very slightly as further dogs are added to the household. (When the continuous variables used to measure the sizes of host or bug populations include fractions, the fractions may be interpreted as the average fraction of time individuals are present in the household.) With additional dogs, more bugs are diverted from feeding on humans because bugs prefer to feed on dogs.

3.11) The human prevalence rate, or fraction of humans infected (Fig. 2I), is positive even in the absence of chickens and dogs because infected humans are initially present in the house. For any fixed number of infected dogs, the human prevalence rate declines very slowly with more chickens. For any fixed number of chickens, the human prevalence rate increases very rapidly as the number of infected dogs increases from 0 to roughly 1.5 and then slowly declines with additional infected dogs. In the study villages, the adult seroprevalence of infection increased from 24% in households with no infected dogs or cats to 48% in households with one to two infected dogs or cats and to 64% with three to five infected dogs or cats (25). The predicted human and bug prevalence rates of *T. cruzi* are both approximately consistent with earlier field data from 1982 and 1984, before the first professional insecticide spraying (9).

To investigate the sensitivity of model predictions to changes in key parameters, Figs. 1 and 2 were recalculated after making three separate changes, one at a time: (i) the maximum number of fourth- and fifth-instar nymphs and adult bugs that the physical infrastructure of the house will support, given an unlimited food supply, was reduced to 150 from the baseline value of 500; (ii) the number of feeding contacts per bug per spring and summer combined was increased to 10 instead of the baseline value of 5; and (iii) the relative preference of bugs for chickens was

increased to 6 while their preference for dogs remained unchanged at the baseline value of 3. The predictions of the model were robust to these changes. The first few infected dogs in the household resulted in a large increase in the number of infected summer bugs, the number of infective feeding contacts per human, and the human prevalence rate. With 10 feeding contacts, keeping chickens in bedroom areas in spring reduced the bug and human prevalence rates even less than when the number of feeding contacts was 5. Reducing the maximum number of bugs from 500 to 150 reduced the human prevalence rate from 0.63 to 0.4.

This model indicates that an increase in the domiciliary chicken population very slightly decreases the human prevalence rate but by an amount that would be undetectable in practice. This marginal benefit for an individual household is accompanied by an increase in the size of the infected summer bug population. Because the bugs are most active and most likely to disperse to other houses as temperatures rise (26), the very slight reduction in the household prevalence rate with more chickens may be outweighed by greater risk of spreading both bug infestation and infection with *T. cruzi* to the rest of the village. These results too are robust with respect to plausible variations of the underlying assumptions of the model.

Keeping domestic animals in bedroom areas entails health and economic hazards, independent of the effect on *T. cruzi* infection. Domestic animals may attract or harbor other potentially dangerous vectors (such as mosquitoes, sandflies, ticks, and lice) and are associated with other pathogens infectious to humans (such as influenza, *Toxocara* spp., and *Echinococcus* spp.). Chickens repeatedly bit by bugs may be less valuable food for householders. Zooprophylactic measures may fail if vectors shift hosts (27).

The model produces a straightforward and clear result from a complex system. Keeping dogs and other highly infectious vertebrates out of bedroom areas can effectively reduce the bug and human prevalence rate, according to the model and field data. Human behavior strongly influences the transmission of *T. cruzi* infection, in addition to chemical and environmental factors that are more commonly emphasized. Low-cost, locally practicable environmental management strategies with intermittent use of insecticides can control human transmission of *T. cruzi* sustainably in rural Argentina and probably elsewhere (28). Historically, multiple simultaneous interventions that included environmental management measures proved successful in controlling malaria (29) and other vector-borne tropical diseases (30). Community education and continuous surveillance through a local health post are key requirements for effective

use of the control strategies identified here.

The task force on applied research on Chagas disease (31) sponsored by the United Nations Development Program, World Health Organization, and World Bank recommends increased efforts to control triatomine vectors that occupy domestic and other habitats in the Andes and Central America. The household model described here could be extended to a spatially explicit form to take account of interactions among households and surrounding forests. A similar modeling approach could prove useful in evaluating zoonophylaxis of other infectious diseases such as malaria, Japanese encephalitis virus, visceral leishmaniasis in Brazil, and the red grouse-hare-louping ill virus system.

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An Autoinhibitory Mechanism for Nonsyntaxin SNARE Proteins Revealed by the Structure of Ykt6p

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Ykt6p is a nonsyntaxin SNARE implicated in multiple intracellular membrane trafficking steps. Here we present the structure of the NH₂-terminal domain of Ykt6p (Ykt6pN, residues 1 to 140). The structure of Ykt6pN differed entirely from that of syntaxin and resembled the overall fold of the actin regulatory protein, profilin. Like some syntaxins, Ykt6p adopted a folded back conformation in which Ykt6pN bound to its COOH-terminal core domain. The NH₂-terminal domain plays an important biological role in the function of Ykt6p, which in vitro studies revealed to include influencing the kinetics and proper assembly of SNARE complexes.

The secretory pathway of eukaryotic cells is comprised of a number of distinct membrane-bound compartments between which proteins and lipids are transported in vesicles that pinch off from one membrane and fuse with another. Targeting and fusion of vesicles is mediated in part by specific interactions between integral membrane proteins on the vesicles and target organelles; these are soluble *N*-ethylmaleimide-sensitive factor attachment receptor proteins, termed v-SNAREs and t-SNAREs, respectively (1, 2). However, a single SNARE can operate in more than one transport step as well as interact with several different SNARE binding partners (3). Previous structural studies have been restricted to the syntaxin SNARE family members (4–11). Here, we determined the atomic structure of Ykt6p, a nonsyntaxin SNARE in solution, by nuclear magnetic resonance (NMR) spectroscopy. We chose Ykt6p because it is an evolutionarily conserved protein that is involved in multiple intracellular transport steps and thus is likely to be subject to regulatory control (12–14).

Extensive experimental trials showed that

full-length Ykt6p is not amenable to structural determination by NMR. Marked improvements in the quality of the NMR spectra were obtained for Ykt6p lacking the COOH-terminal core (the SNARE-SNARE binding domain, residues 141 to 200). The structure of the NH₂-terminal domain of Ykt6p (Ykt6pN, residues 1 to 140) was determined from a total of 2515 NMR-derived restraints (15). The backbone of Ykt6pN was well-defined (Fig. 1A) and consisted of five β strands and four α helices (Fig. 1B). The five β strands were arranged in an β II- β I- β V- β IV- β III antiparallel β sheet, sandwiched by the α A and α B/ α D helices (Fig. 1B). A short α helix (α C) in the linker region connected α B and α D. The overall fold of Ykt6pN was similar to that of the cytoskeletal regulatory protein profilin (16) and is entirely different from the NH₂-terminal domain of the syntaxins [the Habc domain, which forms a three α -helical bundle (4–11)]. Although the structure of full-length Ykt6p could not be determined, elucidation of the relationship between the NH₂- and COOH-domains is essential in order to understand the function of the protein. Using a number of experimental approaches, we directly addressed this relationship.

Inspection of the molecular surface of Ykt6pN revealed a prominent hydrophobic surface on one side of the molecule comprised of several evolutionarily conserved amino acids (Phe31, Phe39, Phe42, and Phe43, see Fig. 1C and Fig. 2, A and D). This

hydrophobic “patch” might represent a protein-binding surface for Ykt6pN, and despite the structural dissimilarities between Ykt6pN and syntaxin Habc, the hydrophobic surface of the core domain of Ykt6p might be a potential binding partner for Ykt6pN. Thus, we compared the ¹H,¹⁵N-HSQC (heteronuclear single-quantum coherence) spectra of Ykt6pN with those of Ykt6pNC (Ykt6pN plus the NH₂-terminal two heptad-repeats of the core domain, residues 1 to 151), and full-length Ykt6p (Fig. 2C). Consistent with a direct interaction between the core domain of Ykt6p and the NH₂-terminal domain, inclusion of the entire core domain resulted in chemical shift changes for a number of amino acid residues in the NH₂-terminal domain of the protein (Fig. 2, B and C). By using a minimal chemical shift perturbation approach (17), the chemical shift changes in the NH₂-domain of Ykt6p that result from interaction with its core domain were summarized (Fig. 2B). Amino acid residues from β III and α A were involved in binding to the core domain of the protein. In contrast, no significant chemical shift changes were observed for residues in β III and α A when Ykt6pN was extended by only two heptad-repeats into the core domain (Ykt6pNC, see Fig. 2C). Thus, the COOH-terminal part of the core domain was in direct contact with β III and α A.

To distinguish between an inter- and an intramolecular basis for the observed interactions between the NH₂- and COOH-terminal domains of Ykt6p, we analyzed the molecular masses of Ykt6p and Ykt6pN using gel-filtration chromatography. Both Ykt6pN and Ykt6p eluted at molecular masses indicative of their being monomers, consistent with an intramolecular interaction between the core domain and the NH₂-terminal domain (Fig. 3A). In addition, gel-filtration chromatography studies revealed that the core domain of Ykt6p (residues 136 to 200) forms a high-molecular-mass aggregate (18). Apparently the core domain in full-length Ykt6p was sequestered, via interaction with its NH₂-domain, thereby preventing it from forming aggregates.

If the hydrophobic surface of Ykt6p (Fig. 2A) were involved in binding to the core domain of the protein, amino acid substitutions at conserved residues in this region would be expected to impair the interaction between the NH₂- and COOH-domains of Ykt6p. Indeed, substitution (19) of a polar

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