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10. The DNAs encoding the COOH-terminal amino acids 857 to 960 (GBR1-CT) and 887 to 921 (GBR1Δ7) of GBR1A were inserted into pGEX-6P-1 (Pharmacia) in-frame with GST [D. B. Smith and K. S. Johnson, *Gene* **67**, 31 (1988)]. The constructs were expressed in *Escherichia coli* XL1blue, and the fusion proteins (GST–GBR1-CT and GST-GBR1Δ7) and GST alone were purified on glutathione-Sepharose beads. The loaded beads were incubated with cytoplasmic extracts of HEK293 cells (*19*) expressing fusion proteins of the Flag epitope with either amino acids 785 to 940 of GBR2 or with amino acids 857 to 960 of GBR1A and processed as described (*6*), except that the FlagM5 antibody (Kodak) was used for probing the immunoblot (1:1000, 1 hour, 25°C). Immunoblots were analyzed by incubation with an anti-mouse secondary antibody conjugated to horseradish peroxidase (Jackson ImmunoResearch Laboratories, 1:5000, 20 min, 25°C) followed by incubation with SuperSignal substrate (Pierce) and exposure to HyperFilm ECL (Amersham Pharmacia Biotech).
11. Total RNA was isolated from the respective rat organs, separated, and blotted according to standard procedures (*7*). A 702–base pair (bp) cDNA probe of GBR2 was labeled with [α-^{32}P]deoxycytidine triphosphate with a random primed labeling kit (Boehringer Mannheim). The blot was hybridized with QuikHyb solution (Stratagene), washed [0.1× sodium chloride/sodium citrate (SSC), 60°C], and exposed to x-ray film.
12. Digoxigenin (Dig)–uridine triphosphate–labeled complementary RNA (cRNA) probes were synthesized from linearized template DNA (Boehringer Mannheim). The open reading frame of GBR1A (*20*) was used for generating sense (1948 bp) and antisense (1751 bp) cRNA probes codetecting transcripts of GBR1A and GBR1B. Sense (1815 bp) and antisense (1763 bp) cRNA probes detecting the GBR2 message were synthesized from the 3′ untranslated region of GBR2. In situ hybridization was performed on frozen brain sections (15 μm) from Wistar rats as described [M. J. Rossner, J. Dorr, P. Gass, M. H. Schwab, K. A. Nave, *Mol. Cell. Neurosci.* **10**, 460 (1997)] with 50 to 100 ng of cRNA probes overnight at 65°C (50% formamide, 5× SSC). Nonspecific binding of probes was eliminated by sequential washes in 2× SSC and in 0.2× SSC at room temperature and at 65°C, and the single-stranded probe was removed by treatment with ribonuclease A (2 μg/ml, 37°C, 30 min). Bound Dig-labeled cRNA probes were detected with alkaline phosphatase–conjugated antibody to Dig. (1:1500 to 1:2000, 1 hour) and standard reagents (Boehringer Mannheim).
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15. Transiently transfected HEK293 cells or untransfected controls were incubated in serum-free medium containing 1 mM 3-isobutyl-1-methyl-xanthine for 30 min at 37°C. Cells were harvested, washed, and resuspended in Krebs-tris buffer (*3*). We treated 500,000 cells per sample in quadruplicates with either GABA (1 mM) or (*R*)-baclofen (500 μM) or respective vehicles for 5 min followed by incubation with forskolin (2 μM) or dimethyl sulfoxide (vehicle) for 20 min at 37°C. Cells were pelleted and boiled for 8 min in tris-EDTA buffer (Amersham Pharmacia Biotech), and cell extracts were assayed for cAMP concentrations with a standard cAMP assay kit (Amersham Pharmacia Biotech).
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17. Transfected cells (*19*) on cover slips were transferred to the stage of an inverted microscope and were continuously perfused with a solution containing 115 mM NaCl, 25 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Hepes, and 10 mM glucose (pH 7.25, adjusted with NaOH). The whole-cell configuration of the patch-clamp technique was applied at room temperature with pipette solutions containing 115 mM K-gluconate, 20 mM KCl, 10 mM Hepes, 5 mM EGTA, 4 mM adenosine triphosphate (Mg^{2+} salt), and 0.3 mM guanosine triphosphate (Na^{2+} salt) (pH 7.25, adjusted with KOH). GABA (100 μM) or (*R*)-baclofen (50 μM) was dissolved in the extracellular solution and was applied steadily to single, lifted cells by a double-barreled application pipette. The inward rectifier currents were activated with voltage ramps. Membrane potentials were corrected for the liquid junction potential (−11 mV). Data collection and analysis were performed as described [G. Köhr and P. H. Seeburg, *J. Physiol.* **492.2**, 445 (1996)].
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19. HEK293 cells (American Type Culture Collection) were cultured in minimal essential medium with Earles's salts supplemented with L-glutamine, 10% fetal calf serum, and 1% Pen/Strep (Gibco-BRL), transiently transfected with standard calcium phosphate–transfection procedures (*7*) with 10 μg of combined cytomegalovirus (CMV) expression plasmids (*20*) per 2 million cells, and used for analysis at 48 hours after transfection.
20. The coding regions for GBR1A (*3*), GIRK1 [Y. Kubo, E. Reuveny, P. A. Slesinger, Y. N. Jan, L. Y. Jan, *Nature* **364**, 802 (1993)], and GIRK2 [M. Stoffel *et al.*, *Biochem. Biophys. Res. Commun.* **212**, 894 (1995)] were amplified from rat brain cDNA with gene-specific primers. The amplicons and the cDNA for GBR2 were subcloned into pBluescript (Stratagene) and also into a CMV expression vector [T. J. Schall *et al.*, *Cell* **61**, 361 (1990)] for transfection in HEK293 cells (*19*).
21. The amino acid sequences were aligned by the clustal method (Lasergene, DNAStar, Madison, WI). Amino acid abbreviations are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Rule Learning by Seven-Month-Old Infants

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A fundamental task of language acquisition is to extract abstract algebraic rules. Three experiments show that 7-month-old infants attend longer to sentences with unfamiliar structures than to sentences with familiar structures. The design of the artificial language task used in these experiments ensured that this discrimination could not be performed by counting, by a system that is sensitive only to transitional probabilities, or by a popular class of simple neural network models. Instead, these results suggest that infants can represent, extract, and generalize abstract algebraic rules.

What learning mechanisms are available to infants on the cusp of language learning? One learning mechanism that young infants can exploit is statistical in nature. For example, Saffran *et al.* (*1*) found that the looking behaviors of 8-month-old infants indicated a sensitivity to statistical information inherent in sequences of speech sounds produced in an artificial language—for example, transitional probabilities, which are estimates of how likely one item is to follow another. In the corpus of sentences "The boy loves apples. The boy loves oranges." the transitional probability between the words "the" and "boy" is 1.0 but the transitional probability between the words "loves" and "apples" is 1/2 = 0.5.

It has been suggested that mechanisms that track statistical information, or connectionist models that rely on similar sorts of information [for example, the simple recurrent network (SRN) (*2*)], may suffice for language learning (*3*). The alternative possibility considered here is that children might possess at least two learning mechanisms, one for learning statistical information and another for learning "algebraic" rules (*4*)—open-ended abstract relationships for which we can substitute arbitrary items. For instance, we can substitute any value of x into the equation $y = x + 2$. Similarly, if we know that in English a sentence can be formed by concatenating any plural noun phrase with any verb phrase with plural agreement, then as soon as we discover that "the three blickets" is a well-formed plural noun phrase and that "reminded Sam of Tibetan art" is a well-

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formed verb phrase with plural agreement, we can infer that "The three blickets reminded Sam of Tibetan art." is a well-formed sentence.

To date, however, there has been no direct empirical test for determining whether young infants can actually learn simplified versions of such algebraic rules. A number of previous experiments drawn from the literature of speech perception (not aimed at the question of rule learning) are consistent with the possibility that infants might learn algebraic rules, but each of these prior experiments could be accounted for by a system that extracted only statistical tendencies. For example, infants who are habituated to a series of two-syllable words attend longer when confronted with a three-syllable word (*5*). An infant who attended longer to a three-syllable word might have noticed a violation of a rule (for example, "all the words here are two syllables"), but an infant could also have succeeded with a statistical device that noted that the three-syllable word had more syllables than the average number of syllables in the preceding utterance. Similarly, Gomez and Gerken (*6*) found that infants who were habituated to a set of sentences constructed from an artificial grammar (VOT-PEL-JIC; PEL-TAM-PEL-JIC) could distinguish between new sentences that were consistent with this grammar (VOT-PEL-TAM-PEL-JIC) from new sentences that were not consistent (VOT-TAM-PEL-RUD-JIC). Such learning might reflect the acquisition of rules, but because all the test sentences were constructed with the same words as in the habituation sentences (albeit rearranged), in these test sentences it was possible to distinguish the test sentence on the basis of statistical information such as transitional probabilities (for example, in the training corpus, VOT was never followed by TAM)—without recourse to a rule.

We tested infants in three experiments in which simple statistical or counting mechanisms would not suffice to learn the rule that was generating the sequences of words. In each experiment, infants were habituated to three-word sentences constructed from an artificial language (*7*) and then tested on three-word sentences composed entirely of artificial words that did not appear in the habituation. The test sentences varied as to whether they were consistent or inconsistent with the grammar of the habituation sentences. Because none of the test words appeared in the habituation phase, infants could not distinguish the test sentences based on transitional probabilities, and because the test sentences were the same length and were generated by a computer, the infant could not distinguish them based on statistical properties such as number of syllables or prosody.

We tested infants with the familiarization preference procedure as adapted by Saffran *et al.* (*1, 8, 9*); if infants can abstract the underlying structure and generalize it to novel words, they should attend longer during presentation of the inconsistent items than during presentation of consistent items.

Subjects were 7-month-old infants, who were younger than those studied by Saffran *et al.* but still old enough to be able to distinguish words in a fluent stream of speech (*8*). In the first experiment, 16 infants were randomly assigned to either an "ABA" condition or an "ABB" condition. In the ABA condition, infants were familiarized with a 2-min speech sample (*10*) containing three repetitions of each of 16 three-word sentences that followed an ABA grammar, such as "ga ti ga" and "li na li." In condition ABB, infants were familiarized with a comparable speech sample in which all training sentences followed an ABB grammar, such as "ga ti ti" and "li na na" (*11*).

In the test phase, we presented infants with 12 sentences that consisted entirely of new words, such as "wo fe wo" or "wo fe fe" (*12*). Half the test trials were "consistent sentences," constructed from the same grammar as the one with which the infant was familiarized (an ABA test sentence for infants trained in the ABA condition and an ABB sentence for infants trained in the ABB condition), and half the test trials were "inconsistent sentences" that were constructed from the grammar on which the infant was not trained (*13*).

We found that 15 of 16 infants showed a preference for the inconsistent sentences (*14*), which was indicated by their looking longer at the flashing side light during presentations of those sentences (*15*) (Table 1).

Although each of the test words in experiment 1 was new, the sequence of phonetic features in the test overlapped to some extent with the sequence of phonetic features in the habituation items. For example, in the ABA condition three habituation sentences contained a word starting with a voiced consonant followed by a word starting with an unvoiced consonant. Each of these three sequences ended with a word that contained a voiced consonant. An infant who was thus expecting the sequence voiced-unvoiced-voiced would be surprised by the inconsistent tests items (each of which was voiced-unvoiced-unvoiced) but not by the consistent items (each of which was voiced-unvoiced-voiced). To rule out the possibility that infants might rely on learning sequences of particular phonetic features rather than deriving a more abstract rule, we conducted a second experiment with the same grammars as in the first experiment but with a more carefully constructed set of words. In experiment 2, then, the set of phonetic features that distinguished the test words from each other did not distinguish the words that appeared in the habituation sentences (*16*). For example, the test words varied in the feature of voicing (for example, if the "A" word was +voiced, the "B" word was −voiced), whereas the habituation words did not vary on the feature of voicing (they were all +voiced). Thus, the habituation items provided no direct information about the relationship between voiced and unvoiced consonants; the same holds for each of the phonetic features that varied in the test items. As in experiment 1, 15 of 16 infants looked longer during the presentation of the inconsistent items than during the presentation of the consistent items (*17*) (Table 1).

Rather than encoding the entire ABA or ABB rule, the infants could have habituated to a single property that distinguishes these grammars. Strings from the ABB grammar contain immediately reduplicated elements (for example, "ti ti"), whereas strings from the ABA grammar do not. In a third experiment, we compared sentences constructed from the ABB grammar with sentences constructed from an AAB grammar (*18, 19*); because reduplication was contained in both grammars, the infants could not distinguish these grammars solely on the basis of information about reduplication (*20*). As in the first two experiments, infants (this time, 16 of 16) looked longer during presentation of the inconsistent items than during presentation of the consistent items (*21*) (Table 1).

Our results do not call into question the existence of statistical learning mechanisms but show that such mechanisms do not exhaust the child's repertoire of learning mechanisms. A system that was sensitive only to transitional probabilities between words

Table 1. Mean time spent looking in the direction of the consistent and inconsistent stimuli in each condition for experiments 1, 2, and 3, and significance tests comparing the listening times. Mean ages of the infants tested were 6 months 27 days (median, 6 months 24 days) in experiment 1, 7 months 1 day (median, 7 months) in experiment 2, and 7 months (median, 7 months 2 days) in experiment 3.

Exp.	Mean listening time (s) (SE)		Repeated measures analysis of variance
	Consistent sentences	Inconsistent sentences	
1	6.3 (0.65)	9.0 (0.54)	$F(14) = 25.7, P < 0.001$
2	5.6 (0.47)	7.35 (0.68)	$F(14) = 25.6, P < 0.005$
3	6.4 (0.38)	8.5 (0.5)	$F(14) = 40.3, P < 0.001$

could not account for any of these results, because all the words in the test sentences are novel and, hence, their transitional probabilities (with respect to the familiarization corpus) are all zero. Similarly, a system that noted discrepancies with stored sequences of words could not account for the results in any of the three experiments, because both the consistent items and the inconsistent items differ from any stored sequences of words. A system that noted discrepancies with stored sequences of phonetic features could account for the results in experiment 1 but not those in experiments 2 and 3. A system that could count the number of reduplicated elements and notice sentences that differ in the number of reduplicated elements could account for the results in experiments 1 and 2, but it could not account for infants' performance in experiment 3.

Likewise, we found in a series of simulations that the SRN is unable to distinguish the inconsistent and consistent sentences, because the network, which represents knowledge in terms of a set of connection weights, learns by altering network connection weights for each word independently (*22*). As a result, there is no generalization to novel words. Such networks can simulate knowledge of grammatical rules only by being trained on all items to which they apply; consequently, such mechanisms cannot account for how humans generalize rules to new items that do not overlap with the items that appeared in training (*23, 24*).

We propose that a system that could account for our results is one in which infants extract abstract algebra-like rules that represent relationships between placeholders (variables), such as "the first item X is the same as the third item Y," or more generally, that "item I is the same as item J." In addition to having the capacity to represent such rules, our results appear to show that infants have the ability to extract those rules rapidly from small amounts of input and to generalize those rules to novel instances. If our position is correct, then infants possess at least two distinct tools for learning about the world and attacking the problem of learning language: one device that tracks statistical relationships such as transitional probabilities and another that manipulates variables, allowing children to learn rules. Even taken together, these tools are unlikely to be sufficient for learning language, but both may be necessary prerequisites.

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7. We leave open the question of whether infants interpreted our materials as genuinely linguistic and thus also leave open the question of whether the mechanisms that acquire abstract rules are specific to language learning or are more generally used in many domains.
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9. Infants sat in a three-sided booth on the laps of their parents (parents wore headphones playing classical music so that they could not hear the stimulus materials) and listened to sounds generated off-line by a speech synthesizer. The booth had a yellow bulb on the center panel; each side panel had a red bulb. A speaker was behind each of the red bulbs. The speakers were connected to a G3 Power Macintosh computer that presented the stimuli and controlled the lights. During the familiarization phase, the yellow light flashed to draw the infant's attention to the center panel of the testing booth while the familiarization speech segment played from both speakers. After the familiarization ended, the infant was presented with test trials. At the beginning of each test trial, the central light was flashed. Once an observer (who also wore headphones playing music to mask the stimuli) indicated that the infant had fixated on the flashing light, the central light was turned off and one of the two side lights began flashing. When the observer indicated that the infant had turned toward the side light, the computer played a three-word test sentence from the speaker that was hidden behind the light, which repeated the test sentence over and over (with a 1.2- to 1.5-s pause between presentations of the test sentence) until either the infant had turned away for two continuous seconds or until 15 s had elapsed. The dependent measure was the total time that the infant spent looking at the light associated with the speaker. Infants who became fussy prior to completion of at least four test trials were not included in the statistical analyses.
10. The first six subjects (three in each condition) were familiarized with 3-min speech samples.
11. The 16 sentences that followed an ABA pattern were "ga ti ga," "ga na ga," "ga gi ga," "ga la ga," "li na li," "li ti li," "li gi li," "li la li," "ni gi ni," "ni ti ni," "ni na ni," "ni la ni," "ta la ta," "ta ti ta," "ta na ta," and "ta gi ta." The 16 sentences that followed the pattern ABB were "ga ti ti," "ga na na," "ga gi gi," "ga la la," "li na na," "li ti ti," "li gi gi," "li la la," "ni gi gi," "ni ti ti," "ni na na," "ni la la," "ta la la," "ta ti ti," "ta na na," and "ta gi gi". Vocalizations of the words used in the above sentences were created with a speech synthesizer, which is available at www.bell-labs.com/project/tts/voices-java.html. The vocalizations were then combined to form the sentences listed above by using a sound editor. A 250-ms pause was placed between consecutive words in each sentence. The sentences were presented in random order and separated by pauses of 1 s.
12. The 12 test trials, which were randomly ordered, included three repetitions of each of four test sentences, two following the ABB pattern ("wo fe fe" and "de ko ko") and two following the ABA pattern ("wo fe wo" and "de ko de").
13. Similar stimuli were used in a study of children's memory and attention [J. V. Goodsitt, P. A. Morse, J. N. Ver Hoeve, *Child Dev.* **55,** 903 (1984)]. That study does not, however, answer our question about rules, because it tested only how well an infant could remember target B in the context of sequences ABA versus AAB versus ABC and not whether infants familiarized with one of those sequences could distinguish it from another.
14. Results for the ABA and ABB conditions were combined, because there was no significant interaction between them, $F(1,14) = 0.15$.
15. Similar results involving transfer from one finite state grammar to another with the same structure but different words have been reported for adult subjects [A. Reber, *J. Exp. Psychol.* **81,** 115 (1969)] and for 11-month-old infants (R. L. Gomez and L.-A. Gerken, paper presented at the Annual Meeting of the Psychonomics Society, Philadelphia, PA, November 1997). These researchers, whose focus was not on rule learning, did not include the phonetic control we introduce in experiments 2 and 3.
16. The 16 habituation sentences that followed the ABA pattern were "le di le," "le je le," "le li le," "le we le," "wi di wi," "wi je wi," "wi li wi," "wi we wi," "ji di ji," "ji je ji," "ji li ji," "ji we ji," "de di de," "de je de," "de li de," "de we de"; ABB items were constructed with the same vocabulary. The test trials were "ba po ba," "ko ga ko" (consistent with ABA), "ba po po," and "ko ga ga" (consistent with ABB).
17. Results for the ABA and ABB conditions were combined, because there was no significant interaction between them, $F(1,14) = 1.95$.
18. In principle, an infant who paid attention only to the final two syllables of each sentence could distinguish the AAB grammar from the ABB grammar purely on the basis of reduplication, but they could not have succeeded in the experiment of Saffran *et al.* (*1*).
19. We thank an anonymous reviewer for suggesting this comparison. The vocabulary used to construct the test and familiarization items was the same as in experiment 2; hence, as in experiment 2, the phonetic features that distinguished the test words from each other did not vary in the habituation items.
20. The ability to extend reduplication to novel words appears to depend on an algebraic rule. To recognize that an item is reduplicated, a system must have the ability to store the first element and compare the second element to the first; the storage, retrieval, and inferential mechanisms that are involved may appear simple but are outside the scope of most neural network models of language and cognition. Conversely, adults are strongly sensitive to the presence of reduplication and its location in phonological constituents [I. Berent and J. Shimron, *Cognition* **64,** 39 (1997)]. For further discussion, see references cited in (*22*).
21. Results for the AAB and ABB conditions were combined, because there was no significant interaction between them, $F(1,14) = 0.002$.
22. The sort of generalizations that such models can draw are dictated by the choice of input representations. If input nodes correspond to words, the model cannot generalize the abstract pattern to new words; if the input nodes correspond to phonetic features, the model cannot generalize to words containing new phonetic features [G. F. Marcus, *Cognition* **66,** 153 (1998); *Cogn. Psychol.*, in press]. An appropriately configured SRN that represented each word by a set of nodes for phonetic features, if it were trained that a voiced consonant followed by an unvoiced consonant was always followed by a voiced consonant, could use memorized sequences of features as a basis to distinguish the test items in experiment 1. However, such a model could not account for the results of experiments 2 and 3, because in those experiments the feature sequences that the network learned about in the familiarization phase would not distinguish the test items.
23. An enhanced version of the SRN [Z. Dienes, G. T. M. Altmann, S. J. Gao, in *Neural Computation and Psychology,* L. S. Smith and P. J. B. Hancock, Eds. (Springer-Verlag, New York, 1995)] aims to model how speakers who are trained on one artificial language are able to learn a second artificial language that has the same structure more rapidly than a second artificial language that has a different structure. This model would not be able to account for our data, however, because the model relies on being supplied with attested examples of sentences that are acceptable in the second artificial language, whereas our infants succeeded in the absence of such information.
24. The problem is not with neural networks per se but with the kinds of network architectures that are currently popular. These networks eschew explicit representations of variables and relationships between variables; in contrast, some less widely discussed neural networks with a very different archi-

tecture do incorporate such machinery and thus might form the basis for learning mechanisms that could account for our data [J. E. Hummel and K. J. Holyoak, *Psychol. Rev.* **104**, 427 (1997)]. Our goal is not to deny the importance of neural networks but rather to try to characterize what properties the right sort of neural network architecture must have.

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Assembly and Analysis of Conical Models for the HIV-1 Core

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The genome of the human immunodeficiency virus (HIV) is packaged within an unusual conical core particle located at the center of the infectious virion. The core is composed of a complex of the NC (nucleocapsid) protein and genomic RNA, surrounded by a shell of the CA (capsid) protein. A method was developed for assembling cones in vitro using pure recombinant HIV-1 CA-NC fusion proteins and RNA templates. These synthetic cores are capped at both ends and appear similar in size and morphology to authentic viral cores. It is proposed that both viral and synthetic cores are organized on conical hexagonal lattices, which by Euler's theorem requires quantization of their cone angles. Electron microscopic analyses revealed that the cone angles of synthetic cores were indeed quantized into the five allowed angles. The viral core and most synthetic cones exhibited cone angles of approximately 19 degrees (the narrowest of the allowed angles). These observations suggest that the core of HIV is organized on the principles of a fullerene cone, in analogy to structures recently observed for elemental carbon.

HIV-1 assembly is initially driven by polymerization of the Gag polyprotein, which forms a spherical shell associated with the inner membrane of the budding particle. The three major regions of Gag all perform essential roles in viral assembly: the NH_2-terminal MA (matrix) region binds the membrane, the central CA (capsid) region mediates important Gag-Gag interactions, and the COOH-terminal NC (nucleocapsid) region packages the viral RNA genome [reviewed in (*1, 2*)]. As the particle assembles, the viral protease cleaves Gag, producing discrete MA, CA, and NC proteins, which subsequently rearrange to form the mature, infectious viral particle. During maturation, MA remains associated with the inner viral membrane, while CA and NC condense about the viral RNA to form an unusual conical structure at the center of the virus (the "core"). The interior of the core is composed of an RNA/NC copolymer, and is surrounded by an outer shell composed of ~1500 copies of CA. The conical core appears to be essential, because Gag mutations that disrupt proper core formation invariably inhibit viral infectivity (*3*). The core probably organizes the viral RNA genome (and its associated enzymes) for uncoating and replication in the new host cell, although these processes are not yet well understood.

Our initial goal was to develop a model system for studying viral core structure and assembly in vitro. The assembly properties of pure recombinant HIV-1 Gag protein fragments have been investigated in several laboratories (*4–7*). Pioneering work by Campbell and Vogt demonstrated that fragments of HIV-1 and Rous sarcoma virus (RSV) Gag proteins that encompass the CA and NC domains can assemble into long hollow cylinders in the presence of RNA (*5*). Building on this, we screened for conditions that would support the assembly of conical (rather than cylindrical) structures. Initially, we employed an HIV-1 RNA template that spanned sites required for genomic RNA packaging (Ψ) and dimerization (DLS), because some models for the viral core have suggested that the genomic RNA dimer dictates the cone morphology (*8*). The protein construct included both the CA and NC domains of HIV-1 Gag, because viral core morphology can be disrupted by mutations in either of these domains, or in the short spacer peptide that connects them (*3*). Finally, solution assembly conditions were varied, because cylinder formation is sensitive to protein and RNA concentrations, salt, and pH (*4–7*). Cone formation was assayed by transmission electron microscopy (TEM) of negatively stained samples.

A mixture of cones (Fig. 1A, arrows) and cylinders formed spontaneously upon incubation of a pure recombinant CA-NC fusion protein with a purified 1400-nucleotide (nt) HIV-1 RNA template in 500 mM NaCl (pH 8.0) (*9*). Cone:cylinder ratios as high as ~2:3 were observed under these optimized conditions. Cones also formed under physiological conditions [that is, 150 mM NaCl (pH 7.2)], albeit at reduced efficiencies. The synthetic cones were capped at both ends, and many appeared strikingly similar to authentic HIV-1 cores (Fig. 1B). Cones formed in vitro varied between 100 and 300 nm in length. Viral cores are typically ~100 nm long (*8, 10*); however, this can also vary considerably because HIV-1 virions range between ~120 to 260 nm in diameter (*11*). These similarities

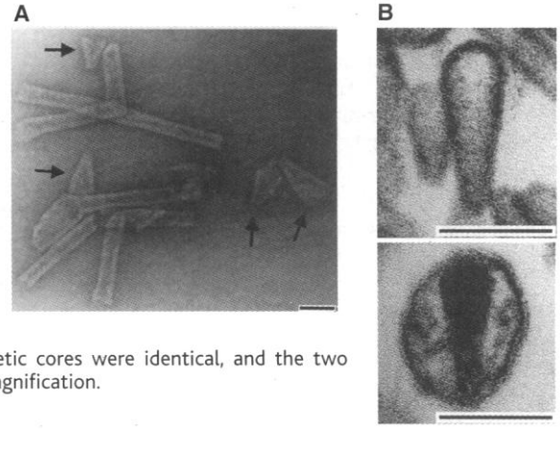

Fig. 1. CA-NC/RNA complexes spontaneously assemble into cones in vitro. (**A**) TEM image of a representative field of negatively stained particles formed by the CA-NC protein on a 1400-nt HIV-1 RNA template. Conical structures are denoted by arrows. Scale bars in Figs. 1, 2, and 4 are 100 nm. (**B**) Selected thin-sectioned TEM images of an authentic HIV-1 virion grown in culture (bottom) and a synthetic CA-NC/RNA cone assembled in vitro (top). Electron microscope preparations of virions and synthetic cores were identical, and the two objects are shown at the same magnification.

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