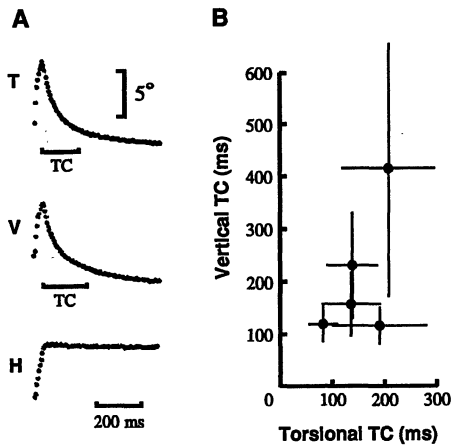

Fig. 4. Time constants (TCs) of exponential drift during unilateral INC inactivation. (**A**) Eye position is plotted as a function of time to illustrate an example of the exponential drift that followed saccades. TCs are illustrated for reference. (**B**) Vertical and torsional time constants of drift from all five animals. Solid circles, mean ±SD for one animal. To obtain this data, we selected an experiment from each animal in which a severe eye position deficit was present ~30 min after muscimol injection. TCs of vertical and torsional drift were then computed for a series of individual examples and averaged.

ing the head front to back). The left-right and clockwise-counterclockwise rotation pairs are mirror images across this plane, but up and down are not (*17*). To achieve bilateral symmetry, up and down must be represented equally on both sides of the brain. This would introduce redundancy, unless the up-down pair is combined with one of the other pairs. Vertical could be combined with horizontal to give two diagonal signals on each side and an independent torsional system. With this system, production of horizontal eye rotations would require coactivation of two muscles per eye, so that their vertical components would cancel out. In the other choice, vertical could be combined with torsional; this combination leaves an independent horizontal system that produces horizontal eye rotations without muscle coactivation. The latter choice is more energy efficient, because in normal orienting behavior, horizontal head and eye rotations are largest and most important, whereas torsional rotations are smallest and are selectively minimized (*4, 10*). Therefore, the oculomotor and vestibular systems appear to utilize the unique coordinate system that optimizes orthogonality, bilateral symmetry, nonredundancy, and energy efficiency.

REFERENCES AND NOTES

1. D. A. Robinson, in *The Control of Eye Movements*, P. Bach-y-Rita, C. C. Collins, J. E. Hyde, Eds. (Academic Press, New York, 1971), pp. 519–538.
2. S. C. Cannon and D. A. Robinson, *J. Neurophysiol.* **57**, 1383 (1987); G. Cheron and E. Godaux, *J. Physiol. (London)* **394**, 267 (1987).
3. D. Tweed and T. Vilis, *J. Neurophysiol.* **58**, 832 (1987).
4. J. D. Crawford and T. Vilis, *ibid.* **65**, 407 (1991).
5. W. M. King *et al.*, *ibid.* **46**, 549 (1981); K. Fukushima *et al.*, *Exp. Brain Res.* **79**, 43 (1990).
6. W. M. King and R. J. Leigh, in *Physiology of Vertical Gaze in Functional Basis of Ocular Motility Disorders*, G. Lennerstrand, D. S. Zee, E. L. Keller, Eds. (Pergamon, Oxford, 1982), pp. 267–276; K. Fukushima, *Prog. Neurobiol.* (N.Y.) **29**, 107 (1987).
7. J. A. Buttner-Ennever and U. Buttner, in *Neuroanatomy of the Oculomotor System*, J. A. Buttner-Ennever, Ed. (Elsevier, Amsterdam, 1988), pp. 137–138; *ibid.*, pp. 149–150.
8. T. Vilis, K. Hepp, U. Schwarz, V. Henn, *Exp. Brain Res.* **77**, 1 (1989).
9. D. Tweed, W. Cadera, T. Vilis, *Vision Res.* **30**, 97 (1990). This method requires three orthogonal magnetic fields to generate three signals in each of two search coils per eye. The resulting six signals are converted into quaternions, which give the axis of rotation from reference position to any other position. The components of eye position quaternions are represented as horizontal, vertical, and torsional eye position for simplicity. These components can be defined as rotation about axes parallel to a head-fixed vertical axis, the interaural axis, and the third orthogonal axis, respectively.
10. D. Tweed and T. Vilis, *ibid.*, p. 111. According to Listing's law, saccades confine eye position to a plane that is orthogonal to the torsional axis (the line of gaze at primary position).
11. Stimulation results require verification because they may reflect the properties of fibers passing through the area, in this case possibly axons from the riMLF.
12. Vestibular imbalance produces unidirectional constant velocity drift.
13. D. A. Robinson, *J. Neurophysiol.* **33**, 393 (1970).
14. The animal with the higher 416-ms vertical time constant died before the optimal INC site was located.
15. A. F. Fuchs, C. R. S. Kaneko, C. A. Scudder, *Annu. Rev. Neurosci.* **8**, 307 (1985).
16. J. H. Anderson, W. Precht, C. Pappas, *Neurosci. Lett.* **14**, 259 (1979); K. Ezure and W. Graf, *Neuroscience* **12**, 85 (1984). For example, the three right canals of frontal- and lateral-eyed animals respond best to rightward, clockwise-upward, and clockwise-downward head rotations.
17. D. A. Robinson, *Biol. Cybern.* **46**, 53 (1982). To envision this point in a concrete way, imagine that up motoneurons were in the right brain and down motoneurons in the left brain. The left motoneuron connections would have to follow a different rule than the right motoneurons, with one side connecting to the elevator muscles of both eyes and the other connecting to the depressors of both eyes. With the organization in Fig. 1, each side excites the inferior muscles of the same body side and the superior muscles of the opposite side.

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Mediation of the Attachment or Fusion Step in Vesicular Transport by the GTP-Binding Ypt1 Protein

NAVA SEGEV

The function of the guanosine triphosphate (GTP)–binding protein Ypt1 in regulating vesicular traffic was studied in a cell-free system that reconstitutes transport from the endoplasmic reticulum to the Golgi. Blocking the Ypt1 protein activity resulted in accumulation of vesicles that act as an intermediate passing between the two compartments. The Ypt1 protein was found on the outer side of these vesicles. The transport process is completed by fusion of these vesicles with the acceptor compartment, and Ypt1 protein activity was needed for this step. Thus, a specific GTP-binding protein is required for either attachment or fusion (or both) of secretory vesicles with the acceptor compartment during protein secretion.

SECRETION OF PROTEINS FROM CELLS requires the orderly progression of those proteins through a series of membranous compartments (*1*). Each step in the transport pathway appears to be mediated by intermediate vesicles that form at the surface of the donor compartment and fuse specifically with the acceptor compartment. As a result, the contents of the vesicles are transported to the acceptor compartment (*2*). The basis for the specificity of fusion of vesicles with the correct acceptor compartment is not known, but multiple GTP-binding proteins may regulate this process at different steps of the pathway (*3*).

Studies of two proteins from yeast, Sec4 and Ypt1, indicate that GTP-binding proteins function in protein transport. Sec4 participates in the last step of secretion. It is localized to the plasma membrane and secretory vesicles in transit to the cell surface and is a GTP-binding protein (*4*). Ypt1 is a 23-kD GTP-binding protein (*5*) that is highly conserved; the yeast and the mammalian proteins share 70% identity (*6*). By genetic and immunolocalization analyses, Ypt1 has been shown to function in transport of proteins at the beginning of the secretory pathway in yeast and in mammalian cells (*3*). The Ypt1 protein is required for protein transport in vitro (*7, 8*). Small GTP-binding proteins have been identified in yeast and mammalian cells (*9*), and five of them have been localized to five different endocytic and secretory organelles (*10*). Association of uncharacterized small GTP-binding proteins with secretory vesicles has also been reported (*11*).

The non-hydrolyzable analog GTPγS blocks protein transport in yeast and mam-

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malian cell-free systems that reconstitute different steps of the secretory pathway (*12–15*). In one system GTPγS was shown to block either the vesicle attachment or fusion step (or both) of transport within the Golgi (*12*), and in another system it blocked the budding step in the formation of post-Golgi secretory vesicles (*15*). However, the specific proteins affected by GTPγS have not been identified.

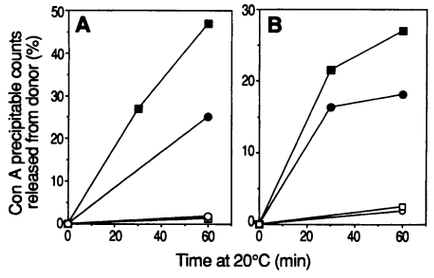

Fig. 1. Accumulation of putative intermediate vesicles in transport reactions in which activity of Ypt1 is blocked. (**A**) Inhibition by anti-Ypt1. Comparison of vesicle formation in the presence (squares) or absence (circles) of anti-Ypt1. Donor microsomes were incubated in the presence of anti-Ypt1 (20 μg of Fab fragment, an amount sufficient to cause a complete block of transport), cytosol, and phs, with ATP (closed symbols) and without ATP (open symbols). Vesicle formation was equated with the amount of core-glycosylated ^{35}S-labeled pro-α factor (Con A precipitable labeled protein) remaining in the supernatant after donor microsomes were separated by centrifugation (*24*). (**B**) Inhibition by mutation of *ypt1*. Donor microsomes and phs fractions were prepared from a *ypt1-1* mutant strain (NSY12). Cytosols were prepared from mutant (squares) and wild-type (GPY60, circles) cells. Vesicle formation was measured as described in (A), after incubation with (closed symbols) or without (open symbols) ATP. Results shown are typical of three experiments; the percent of vesicle accumulation varied between 35 to 50% in different experiments.

To determine whether Ypt1 functions in one of the substeps, budding or attachment and fusion, the effect of specifically blocking the activity of this protein on protein transport in vitro was studied. The activity of Ypt1 was inhibited either by a specific antibody to Ypt1 (anti-Ypt1) or by a mutation, and accumulation of intermediate vesicles in the transport reaction was monitored. The yeast cell-free system used reconstitutes protein transport from the endoplasmic reticulum (ER) to the Golgi complex and is dependent on Ypt1 activity (*7*). The transport process was followed by analyzing the processing of ^{35}S-labeled pro-α factor (*24*). When first translocated into the ER (donor compartment) the pro-α factor becomes core-glycosylated; when it is transported into the Golgi (acceptor) it is modified further by outer chain glycosylation (*7, 13*). The labeled pro-α factor in the putative intermediate vesicles should be identical to that found in the donor compartment. Intermediate vesicles are expected to be of smaller size and were separated from the donor compartment by differential centrifugation. Such a vesicular intermediate was isolated recently from unblocked reactions with a similar in vitro system (*16*).

When transport was completely blocked by anti-Ypt1, putative intermediate vesicles formed by the donor compartment accumulated. The putative intermediate vesicles, like the donor compartment, contained ^{35}S-labeled core-glycosylated α factor but, unlike the donor compartment, stayed in the supernatant after centrifugation. The formation of these vesicles was dependent on the presence of adenosine triphosphate (ATP) (Fig. 1A) and was most efficient when two other fractions that are needed for the transport reaction, cytosol and pellet from high-speed centrifugation (phs), were present (*17*). The putative intermediate vesicles were present but less abundant in reactions that were not blocked with anti-Ypt1 (Fig. 1A). Since these vesicles were detected in unblocked reactions and the components of the in vitro reaction required for their formation were identical to those necessary for transport as well as for vesicle formation in this and other systems (*13, 16*), they appear to be naturally occurring structures.

An alternative way to block the Ypt1 protein activity is by mutation. Extracts from cells carrying the mutation *ypt1-1* are defective in transport, but transport can be restored if a cytosolic fraction from wild-type cells is provided (*7*). In transport reactions performed with mutant components, putative intermediate vesicles accumulated (Fig. 1B). Thus, reactions containing mutant *ypt1* cell extracts were blocked at some point after formation of the vesicles. The accumulation of vesicles in reactions containing extracts of *ypt1-1* cells was greater than in reactions containing wild-type cytosol. In both cases the accumulation was ATP-dependent.

These data show that a putative intermediate is released from the donor compartment during transport reactions, and this intermediate accumulates in reactions blocked for Ypt1 protein activity. The next set of experiments characterizes the putative

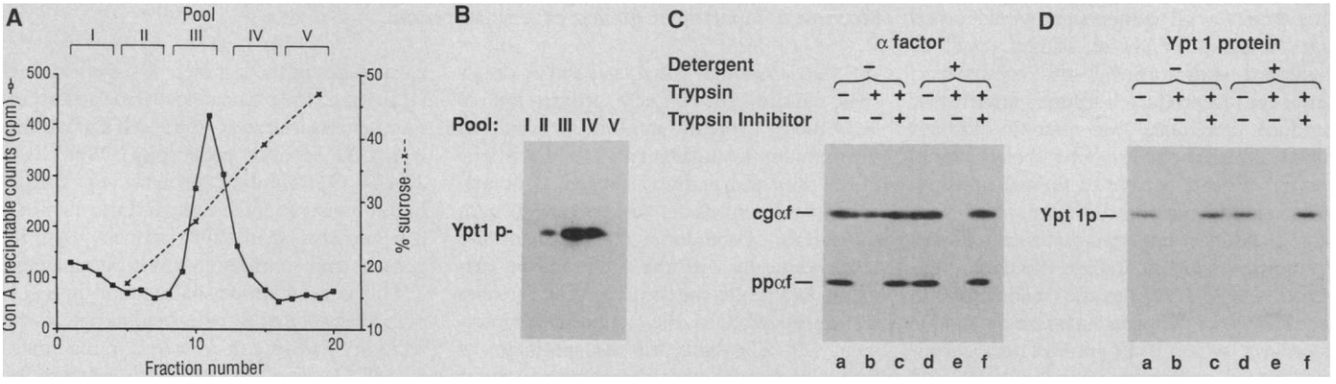

Fig. 2. Characterization of the intermediate as vesicles with Ypt1 protein at the surface. (**A**) Sedimentation analysis of the intermediate vesicles. The fraction containing intermediate vesicles from a total of four transport reactions (50 μl each) was isolated in the presence of anti-Ypt1 as described in Fig. 1. The sample was applied to the top of a 10 to 50% (w/w) linear sucrose gradient (in 10 mM Hepes, pH 6.8). The gradient was centrifuged at 150,000*g* for 140 min and fractionated from the top. The amount of core-glycosylated pro-α factor (Con A precipitable radioactivity) in each fraction was an indication of the location of the vesicles. (B) Ypt1 protein is associated with the intermediate vesicles. Fractions were pooled into five sets of four, diluted with an equal volume of reaction buffer, and centrifuged (200,000*g* for 1 hour). The P200 pellet was resuspended in reaction buffer (*13*) and subjected to SDS polyacrylamide gel electrophoresis (PAGE) and immunoblotting with affinity purified anti-Ypt1 (*3*). (**C**) The containment of core-glycosylated pro-α factor within sealed membranes. Intermediate vesicles were isolated and treated with protease as described (*25–27*). After the protease reaction, half of each sample was subjected to immunoprecipitation with antibody to α factor, SDS PAGE, and fluorography (cgαf, core glycosylated pro-α factor; pp-αf, pre–pro-α factor). (**D**) Detection of Ypt1 protein on the cytoplasmic side of the intermediate vesicle membrane. After the protease reaction (*25–27*) half of each sample was subjected to SDS PAGE and immunoblotting with anti-Ypt1. The lower band, seen in the lanes in which the protease was active (Fig. 2D, lanes b and e), probably represents a degradation product.

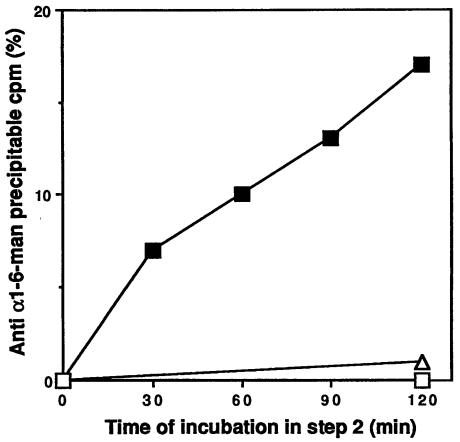

Fig. 3. Consumption of the intermediate, accumulated in the presence of anti-Ypt1, after addition of Ypt1. In step 1, the intermediate was isolated after incubation of a transport reaction in the presence of anti-Ypt1 (Fig. 1; 60 min at 20°C). In step 2 the conversion of core-glycosylated to outer-chain glycosylated pro-α factor [detected by immunoprecipitation with antiserum to α1-6-linked mannose (anti-α1-6-man)] was measured after incubation at 20°C in the presence of Ypt1 (12.5 μg), ATP, and fresh phs (closed squares). Control reactions were incubated in the absence of Ypt1 (triangles) or ATP (open squares). The results are typical of three experiments. The percent of outer-chain glycosylation in step 2 varied between 15 to 30% in different experiments and was similar to the percentage of transport in unblocked reactions in the same experiment. Ypt1 was purified from *Escherichia coli* cells containing the plasmid pRS9 (7). The amount of Ypt1 added during step 2 can completely relieve the inhibition of transport by anti-Ypt1 if added during step 1.

intermediate that accumulates in the Ypt1-blocked reactions as transport vesicles, similar to the intermediate vesicles that have been isolated from unblocked reactions (*16*). To determine whether the vesicles that accumulated in Ypt1-blocked reactions, were of homogeneous size, sedimentation analysis was performed. When the putative intermediate vesicle fraction was analyzed on a velocity sucrose gradient, the vesicles containing core-glycosylated α factor migrated in a single sharp peak (Fig. 2A). In an equilibrium sucrose gradient, the vesicle fraction migrated to a density that was less than that of the donor compartment (37.5 and 42% sucrose, respectively) (*17*). The core-glycosylated α factor in the intermediate fraction was protected from protease digestion in the absence, but not the presence, of detergent (Fig. 2C). The unglycosylated α factor (lower band), which was not translocated into the microsomes and is present in these samples, was sensitive to the protease in the absence of detergent. This result suggests that the core-glycosylated α factor in this fraction is contained within sealed membranes. The sealed membranes, isolated from Ypt1-blocked reactions, are apparently a real intermediate and not artificially broken microsomes, because an ER marker, 3-hydroxy-3-methylglutaryl coenzyme A reductase (Hmg1) (*18*), was not present in this fraction. The release of core-glycosylated α factor [concanavalin A (Con A) precipitable material] from the donor compartment was compared to that of Hmg1 (determined by immunoblot analysis). While 35% of the α factor was released to the intermediate compartment during the incubation (1 hour, 20°C), release of Hmg1 was not detected (*17*). These results confirm that the intermediate accumulated in the Ypt1-blocked reactions consists of a population of membranous vesicles that are distinct from the donor compartment not only by their size, but also by their composition.

If Ypt1 participates in vesicular transport, it would be found on the outer side of the vesicles. The Ypt1 was present in all the fractions that were used for the in vitro assay (7). Immunoblot analysis on the intermediate vesicle fraction, which had been sedimented on a velocity sucrose gradient, indicated that Ypt1 was present in this fraction. The presence of Ypt1 protein in the pellet (P200) of the pooled fractions coincided with the presence of the core-glycosylated α factor (Fig. 2B). Because the core-glycosylated α factor marks the vesicles, it can be concluded that Ypt1 is present in the vesicle fraction. The Ypt1 in the intermediate fraction was sensitive to protease even in the absence of detergent (Fig. 2D). Under these conditions the vesicles must have been sealed since the core-glycosylated α factor present in this fraction was protected (Fig. 2C). These results demonstrate that Ypt1 is present on the outer side of transport vesicles and consistent with its function in directing vesicular traffic.

Because vesicles accumulate in Ypt1-blocked reactions, Ypt1 seems to be required for their consumption. To verify that this is the case, and to show that the vesicles accumulated in the Ypt1-blocked reactions are capable of completing the transport process, an attempt was made to reverse the effect of the antibody by addition of excess of Ypt1 protein. The Ypt1 protein produced in bacteria can alleviate inhibition of transport by anti-Ypt1 when added at the beginning of the reaction (7). The reversal experiments were done in two steps. (i) The intermediate was allowed to accumulate during incubation of a transport reaction in the presence of anti-Ypt1. The intermediate was then isolated from the donor compartment by centrifugation. (ii) The intermediate was incubated in the presence of an excess of Ypt1 protein. The amount of intermediate that fused with the acceptor compartment was determined by measuring outer chain glycosylated α-factor. During the second incubation the amount of outer chain–glycosylated α factor increased, and this increase was dependent on the presence of both excess Ypt1 protein and ATP (Fig. 3). Thus, the intermediate that accumulated during reactions blocked by anti-Ypt1 is capable of completing the transport process and Ypt1 protein activity is needed for the fusion of the intermediate with the acceptor compartment. Addition of the phs fraction, but not the microsome fraction, during the second incubation increased consumption of the intermediate (*17*). Thus, the microsome fraction serves as the donor compartment and the phs fraction contains the acceptor compartment. The fact that the phs fraction was also needed for efficient vesicle formation suggests that this crude membrane fraction also contains factors required for the budding of the vesicles. Alternatively, it is possible that the reaction that was subdivided is actually an early step occurring within the Golgi and that the membranes included in the phs fraction are required for completing the transport from ER to the first Golgi cisterna (see below). The consumption of the intermediate vesicles was sensitive to the addition of a Ca^{2+} chelator (5 mM EGTA) (*17*), consistent with previous results that Ypt1 and Ca^{2+} function independently in protein transport (7).

In summary, Ypt1 functions in protein transport at a step after the formation of intermediate vesicles, for example, in the attachment or fusion of the vesicles with the acceptor compartment. The Ypt1 protein appears to function early in the secretory pathway (*3*, 7). However, it is not clear at which step it participates and whether it functions in only one step. Genetic analysis of yeast *ypt1* mutants indicates that it acts in either the ER to Golgi step (*19*) or in transport within the early Golgi compartments (*3*); immunolocalization in mammalian cells suggested its function in the Golgi (*3*, *20*). The in vitro system reconstituting transport from the ER to the Golgi requires Ypt1 activity and was suggested to carry transport from ER to the second compartment of the Golgi complex (*21*). Other in vitro studies suggested that Ypt1 functions within the Golgi (*8*). Thus, it is possible that Ypt1 functions either between ER and the Golgi or within the early Golgi cisterna, or in both steps.

GTP-binding proteins might function in secretion in a manner similar to that of either the signal transducing G proteins or the elongation factors of protein transport. Bourne (*22*) suggested the analogy between GTP-binding proteins and elongation factor Tu and emphasized the possible role of GTP-hydrolysis in assuring unidirectional-

ity. Also, GTP-binding proteins might increase accuracy of protein sorting. Coupling GTP hydrolysis with vesicle fusion might serve as a proofreading mechanism (7), similar to that proposed for elongation factor Tu in protein translation (*23*).

REFERENCES AND NOTES

1. G. Palade, *Science* **189**, 347 (1975).
2. S. R. Pfeffer and J. E. Rothman, *Annu. Rev. Biochem.* **56**, 829 (1987).
3. N. Segev, J. Mulholland, D. Botstein, *Cell* **52**, 915 (1988).
4. P. Novick, C. Field, R. Schekman, *ibid.* **21**, 205 (1980); B. Goud, A. Salminen, N. C. Walworth, P. Novick, *ibid.* **53**, 753 (1988); A. Salminen and P. J. Novick, *ibid.* **49**, 527 (1987).
5. D. Gallwitz, C. Donath, C. Sander, *Nature* **306**, 707 (1983); H. D. Schmitt, P. Wagner, E. Pfaff, D. Gallwitz, *Cell* **47**, 401 (1986).
6. H. Haubruck, C. Disela, P. Wagner, D. Gallwitz, *EMBO J.* **6**, 4049 (1987); N. Touchot, P. Chardin, A. Tavitian, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 8210 (1987).
7. D. Baker, L. Wuestehube, R. Schekman, D. Botstein, N. Segev, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 355 (1990).
8. R. A. Bacon, A. Salminen, H. Ruohola, P. Novick, S. Ferro-Novick, *J. Cell Biol.* **109**, 1015 (1989).
9. R. D. Burgoyne, *Trends Biochem. Sci.* **14**, 394 (1989).
10. P. Chavrier, R. G. Parton, H. P. Hauri, K. Simons, M. Zerial, *Cell* **62**, 317 (1990); B. Goud, A. Zahraoui, A. Tavitian, J. Saraste, *Nature* **345**, 553 (1990); F. Darchen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5692 (1990); G. Fischer v. Mollard *et al.*, *ibid.*, p. 1988.
11. R. D. Burgoyne and A. Morgan, *FEBS Lett.* **245**, 122 (1989); J. P. Doucet, S. Fournier, M. Parulekar, J. M. Trifaro, *ibid.* **247**, 127 (1989); G. M. Widey, M. J. Im, C. J. Homcy, R. M. Graham, *J. Cell Biol.* **109**, 293 (1989); J. Rubins, T. M. Shannon, B. F. Dickey, *ibid.*, p. 298; P. J. Padfield and J. D. Jamieson, *ibid.*, p. 299.
12. P. Melancon *et al.*, *Cell* **51**, 1053 (1987).
13. D. Baker, L. Hicke, M. Rexach, M. Schleyer, R. Schekman, *ibid.* **54**, 335 (1988).
14. H. Ruohola, A. K. Kabcenell, S. Ferro-Novick, *J. Cell Biol.* **107**, 1465 (1988); C. J. M. Beckers and W. E. Balch, *ibid.* **108**, 1245 (1989); L. S. Mayorga, R. Diaz, P. D. Stahl, *Science* **244**, 1475 (1989); Y. Goda and S. R. Pfeffer, *Cell* **55**, 309 (1988).
15. S. A. Tooze, U. Weiss, W. B. Huttner, *Nature* **347**, 207 (1990).
16. M. E. Groesch, H. Ruohola, R. Bacon, G. Rossi, S. Ferro-Novick, *J. Cell Biol.* **111**, 45 (1990).
17. N. Segev, unpublished data.
18. R. Wright, M. Basson, L. D'Ari, J. Rine, *J. Cell Biol.* **107**, 101 (1988).
19. H. D. Schmitt, M. Puzicha, D. Gallwitz, *Cell* **53**, 635 (1988).
20. G. Jedd and N. Segev, unpublished data.
21. A. Franzusoff and R. Schekman, *EMBO J.* **8**, 2695 (1989).
22. H. R. Bourne, *Cell* **53**, 669 (1988).
23. J. J. Hopfield, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4135 (1974); R. C. Thompson, *Trends Biochem. Sci.* **13**, 91 (1988).
24. The microsome-based transport reaction contained microsomes, phs, and cytosol. Their preparation and the transport reaction were described (7). A typical transport reaction contained microsomes (2 μg), phs (20 μg), and cytosol (100 μg). The intermediate vesicle fraction was the supernatant recovered after centrifugation at 14,000*g* (10 min at 4°C). The *Saccharomyces cerevisiae* strains used were: GPY60 (*Matα, ura3-52, trp1, leu2, his4, pep4::URA3*); NSY12 (*Matα, ura3-52, trp1, leu2, his4, pep4::URA3, ypt1-1*). Anti-Ypt1 Fab fragments were prepared from anti-trpE-Ypt1 as described (7). The inhibition by the antibody was shown to be specific for Ypt1 (*3*, 7). The analysis of core- and outer chain-glycosylated pro-α factor and the materials used for the in vitro system were described (*7*, *13*).
25. Protease protection experiment (Fig. 2, C and D): Intermediate was isolated after incubation of a transport reaction in the presence of anti-Ypt1 (as described in Fig. 1). The samples were then treated with trypsin (50 μg/ml) for 1 hour at 4°C in the presence or absence of detergent (Triton X-100, 0.1%). The protease reaction was terminated by the addition of trypsin inhibitor (100 μg/ml). SDS gel electrophoresis (11%) and Laemmli buffer system (*26*) were used for all protein electrophoresis. Proteins were electroblotted to nitrocellulose and radiographic detection was performed with affinity-purified antibodies and ^{125}I-labeled protein A (*27*).
26. U. K. Laemmli, *Nature* **227**, 680 (1970).
27. W. N. Burnette, *Anal. Biochem.* **112**, 195 (1981).

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Potential of Visual Cortex to Develop an Array of Functional Units Unique to Somatosensory Cortex

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The identification of specialized areas in the mammalian neocortex, such as the primary visual or somatosensory cortex, is based on distinctions in architectural and functional features. The extent to which certain features that distinguish neocortical areas in rats are prespecified or emerge as a result of epigenetic interactions was investigated. Late embryonic visual cortex transplanted to neonatal somatosensory cortex was later assayed for "barrels," anatomically identified functional units unique to somatosensory cortex, and for boundaries of glycoconjugated molecules associated with barrels. Barrels and boundaries form in transplanted visual cortex and are organized in an array that resembles the pattern in the normal barrelfield. These findings show that different regions of the developing neocortex have similar potentials to differentiate features that distinguish neocortical areas and contribute to their unique functional organizations.

THE FUNCTIONAL ORGANIZATION OF the mammalian neocortex is based on its parcellation into numerous anatomically and functionally distinct areas (*1*). In spite of interest in the processes that control the differentiation of neocortical areas during development (*2*, *3*) and throughout evolution (*4*), fundamental issues remain unresolved. Adult neocortical areas can be distinguished by differences in their connections and by their architectural features. These differences underlie the unique functional properties of each area and are related to the anatomically identified functional units. These specializations include the "blobs" of the primary visual area of primates (*5*) and the "barrels" of the primary somatosensory area of rodents (*6*). However, the developing neocortex lacks many of the features that distinguish adult neocortical areas. Early in neocortical development, but after the generation and migration of cortical neurons, cytoarchitecture is uniform between areas that will later have an abrupt border in the adult (7); major classes of projection neurons are not restricted to their limited distributions across neocortical areas as in adults (*8*); and functional units are only beginning to emerge (*9*, *10*). To define the mechanisms responsible for the differentiation of neocortical areas, it is necessary to determine the extent to which each area's characteristics are prespecified or are caused by extrinsic influences that operate as the cortex is assembled.

To address this issue, we used the primary somatosensory area of rodent parietal cortex, which contains discrete functional units, termed "barrels." Barrels are isolated aggregates of layer 4 neurons, innervated by clusters of ventrobasal thalamic afferents, and arranged in a pattern isomorphic to that of the sensory hairs on the rodent's body surface (*10*). Therefore, barrels are not only indicative of the functional organization of the rodent's somatosensory cortex but are also an example of the architectural differentiation that distinguishes neocortical areas. Barrels develop gradually from a uniform cortical plate midway through the first week after birth (*10*, *11*). Experimental studies have demonstrated that the formation of barrels depends on input from an intact sensory periphery during a critical period of development (*10*, *12*). Although these studies demonstrated that barrel formation can be modified, they did not address whether

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