establish whether this protein is present in other human bladder carcinoma cells or in other naturally occurring tumours. Such an approach may help in determining whether the T24 oncogene has an aetiological role in human neoplasia.

Detection of dominant human transforming genes by DNA-mediated gene transfer techniques has generally required the use of the NIH/3T3 cell line as the recipient assay cell[12-16,18,19]. As NIH/3T3 is a continuous, heteroploid cell line[38], it is possible that genes which transform this cell line might be unable to transform normal cells. It is known that both v-*bas* and v-*ras* are capable of inducing a variety of malignancies in animals[39-41]. Thus, it seems likely that when the human cellular homologue of these *onc* genes is activated, as in the case of the T24 oncogene, this gene would possess the capacity to transform normal cells as well.

The number of different genes that are activated as dominant transforming genes in human tumours is not known[12-19]. DNAs isolated from carcinomas of the colon and pancreas as well as from a fibrosarcoma and a rhabdomyosarcoma have been shown to transform NIH/3T3 cells in transfection assays (ref. 19 and M.B. *et al.*, unpublished). Although none of these putative human oncogenes has been found to correspond to the human homologues of retroviral *onc* genes used in the present studies, our findings that c-*bas* (human) is a nontransforming allele of the T24 oncogene suggest that additional relationships between human oncogenes and retroviral *onc* sequences may be uncovered.

It has been well established that the cellular sequences transduced by retroviruses require viral genetic information in order to induce malignancy. The present studies demonstrate that at least one of these cellular sequences, c-*bas* (human), can be activated by a mechanism not requiring recombination with retroviral sequences. Detailed restriction enzyme analysis of this gene and its transforming allele, the T24 oncogene, has not revealed detectable differences. Thus, we must conclude that subtle genetic changes have caused the activation of this gene. Comparative sequence analysis of the T24 oncogene and c-*bas* (human) will ultimately define, not only the nature, but the number of genetic changes that led to the acquisition of transforming activity by c-*bas* (human) in T24 bladder carcinoma cells.

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1. *Cold Spring Harb. Symp. quant. Biol.* **44,** pt 2 (1980).
2. Frankel, A. E., Gilbert, J. H., Porzig, K. J., Scolnick, E. M. & Aaronson, S. A. *J. Virol.* **30,** 821–827 (1979).
3. Shibuya, M., Hanafusa, T., Hanafusa, H. & Stephenson, J. R. *Proc. natn. Acad. Sci. U.S.A.* **77,** 6536–6540 (1980).
4. Andersen, P. R. *et al. Cell* **26,** 129–134 (1981).
5. Barbacid, M., Breitman, M. L., Lauver, A. L., Long, L. K. & Vogt, P. K. *Virology* **110,** 411–419 (1981).
6. Beemon, K. *Cell* **24,** 145–153 (1981).
7. Wang, L. H. *et al. J. Virol.* **40,** 258–267 (1981).
8. Shih, C., Shilo, B. Z., Goldfarb, M. P., Dannenberg, A. & Weinberg, R. A. *Proc. natn. Acad. Sci. U.S.A.* **76,** 5714–5718 (1979).
9. Cooper, G. M., Okenquist, S. & Silverman, L. *Nature* **284,** 418–421 (1980).
10. Cooper, G. M. & Neiman, P. E. *Nature* **287,** 656–659 (1980).
11. Hopkins, N., Besmer, P., DeLeo, A. B. & Law, L. W. *Proc. natn. Acad. Sci. U.S.A.* **78,** 7555–7559 (1981).
12. Lane, M. A., Sainten, A. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* **78,** 5185–5189 (1981).
13. Shih, C., Padhy, C., Murray, M. & Weinberg, R. A. *Nature* **290,** 261–264 (1981).
14. Krontikis, T. G. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* **78,** 1181–1184 (1981).
15. Murray, M. J. *et al. Cell* **25,** 355–361 (1981).
16. Perucho, M. *et al. Cell* **27,** 467–476 (1981).
17. Smith, B. L., Anisowicz, A., Chodosh, L. A. & Sager, R. *Proc. natn. Acad. Sci. U.S.A.* **79,** 1964–1968 (1982).
18. Lane, M. A., Sainten, A. & Cooper, G. M. *Cell* **28,** 873–880 (1982).
19. Pulciani, S. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 2845–2849 (1982).
20. Bubenik, J. *et al. Int. J. Cancer* **11,** 765–773 (1973).
21. Marshall, C. J., Franks, L. M. & Carbonell, A. W. *J. natn. Cancer Inst.* **58,** 1743–1747 (1977).
22. Goldfarb, M., Shimizu, K., Perucho, M. & Wigler, M. *Nature* **296,** 404–409 (1982).
23. Shih, C. & Weinberg, R. A. *Cell* **29,** 161–169 (1982).
24. Lautenberger, J. A., Schulz, R. A., Garon, C. F., Tsichlis, P. N. & Papas, T. S. *Proc. natn. Acad. Sci. U.S.A.* **78,** 1518–1522 (1981).
25. Souza, L. M., Komaromy, M. C. & Baluda, M. A. *Proc. natn. Acad. Sci. U.S.A.* **77,** 3004–3008 (1980).
26. Shalloway, D., Zelentz, A. D. & Cooper, G. M. *Cell* **24,** 531–541 (1981).
27. Srinivasan, A., Reddy, E. P. & Aaronson, S. A. *Proc. natn. Acad. Sci. U.S.A.* **78,** 2077–2081 (1981).
28. Vande Woude, G. F. *et al. Proc. natn. Acad. Sci. U.S.A.* **76,** 4464–4468 (1979).
29. Tronick, S. R. *et al. Proc. natn. Acad. Sci. U.S.A.* **76,** 6314–6318 (1979).
30. Sherr, C. J., Fedele, L. A., Oskarsson, M., Maizel, J. & Vande Woude, G. F. *J. Virol.* **34,** 200–212 (1980).
31. Robbins, K. C., Devare, S. G. & Aaronson, S. A. *Proc. natn. Acad. Sci. U.S.A.* **78,** 2918–2922 (1981).
32. Santos, E., Pulciani, S. & Barbacid, M. *Fedn Proc.* (in the press).
33. Eva, A. *et al., Nature* **295,** 116–119 (1982).
34. Lawn, R. M., Fritsch, E. F., Parker, R. C., Blake, G. & Maniatis, T. *Cell* **15,** 1157–1174 (1978).
35. Shih, T. Y., Weeks, M. O., Young, H. A. & Scolnick, E. M. *Virology* **96,** 64–79 (1979).
36. Aaronson, S. A. & Todaro, G. J. *Virology* **36,** 254–261 (1968).
37. Rasheed, S., Nelson-Rees, W. A., Toth, E. M., Arnstein, P. & Gardner, M. B. *Cancer* **33,** 1027–1033 (1974).
38. Jainchill, J. L., Aaronson, S. A. & Todaro, G. J. *J. Virol.* **4,** 549–553 (1969).
39. Peters, R. L., Rabstein, L. S., Van Vleck, R., Kelloff, G. J. & Huebner, R. J. *J. natn. Cancer Inst.* **53,** 1725–1731 (1974).
40. Harvey, J. J. *Nature* **204,** 1104–1107 (1964).
41. Scher, C. D., Scolnick, E. M. & Siegler, R. *Nature* **256,** 225–228 (1975).
42. Southern, E. M. *J. molec. Biol.* **98,** 503–517 (1975).
43. Davis, R. W., Simon, M. & Davidson, N. *Meth. Enzym.* **21,** 413–428 (1971).
44. Cox, R. A. *Meth. Enzym.* **12,** 120–129 (1967).
45. Aviv, H. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **69,** 1408–1412 (1972).
46. Bailey, J. M. & Davidson, N. *Analyt. Biochem.* **70,** 75–85 (1976).
47. Barbacid, M., Lauver, A. L. & Devare, S. *J. Virol.* **33,** 196–207 (1980).
48. Bonner, W. M. & Laskey, R. A. *Eur. J. Biochem.* **46,** 83–88 (1974).

Synthesis and assembly of hepatitis B virus surface antigen particles in yeast

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The surface antigen of hepatitis B virus (HBsAg) has been synthesized in the yeast Saccharomyces cerevisiae *by using an expression vector that employs the 5′-flanking region of yeast alcohol dehydrogenase I as a promoter to transcribe surface antigen coding sequences. The protein synthesized in yeast is assembled into particles having properties similar to the 22-nm particles secreted by human cells.*

HEPATITIS B virus[1-3] is a 42-nm particle (the Dane particle) consisting of a core containing the viral genome (~3,200 base pairs (bp) of partially single-stranded DNA[4]) bound to the core protein and its own DNA polymerase[5-9]; the virus core is surrounded by a phospholipid-containing envelope carrying the major surface antigenic determinants (HBsAg). These seem to

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reside mainly in a single protein, which occurs in both a glycosylated and non-glycosylated form (molecular weight (M_r) 27,000–29,000 and 23,000–25,000, respectively, as measured on SDS gels[10,11]). Infection with hepatitis B virus (HBV) leads not only to the production of Dane particles but also to a dramatic overproduction of 22-nm large particles and filaments (the HBsAg particle) that contain the elements of the surface envelope. These HBsAg particles are about 1,000-fold more immunogenic than the unassembled HBsAg protein[12]. Because of its narrow host range (humans and chimpanzees), and as it cannot be propagated in tissue culture, investigation of the structure and mechanism of infection of HBV has been severely restricted. The advent of molecular cloning has allowed the mplete virus genome to be cloned and sequenced[5–7,13–15], and the coding regions for the different antigens have been identified[5–7], raising the possibility of expressing HBV genes in alternative host systems. However, in previous attempts, high level production of HBsAg-related immunogenic material was not achieved in bacteria even in systems using powerful bacterial promoters[14,16,17]. We therefore examined yeast as an alternative host system; this organism has complex membrane systems and the ability to secrete and glycosylate proteins by processes resembling those observed in higher cells[18]. Recently, yeast has also been developed to give a system which allows the introduction, maintenance and expression of foreign genes[19–21]. Here we report the construction of an autonomously replicating plasmid containing HBsAg-coding sequences linked to the yeast alcohol dehydrogenase I promoter. Yeast transformed with these vectors synthesize and accumulate particulate material that specifically reacts with antibodies against HBsAg.

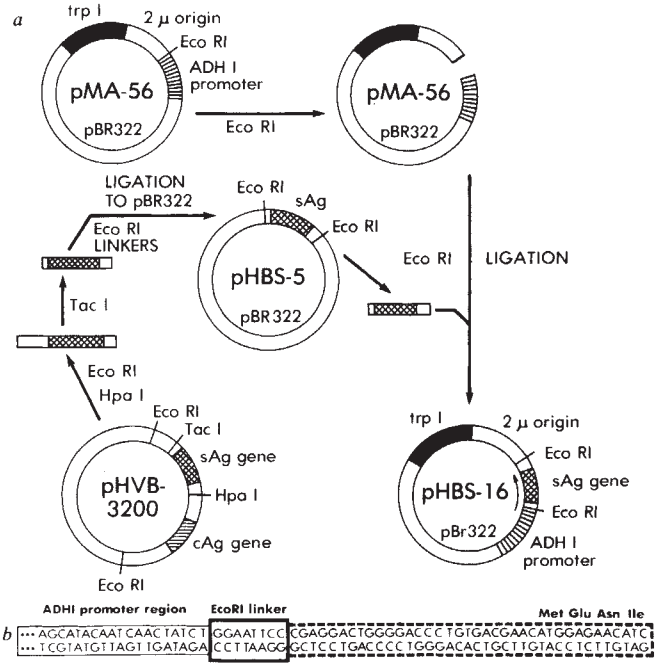

Fig. 1 *a*, Construction of plasmids for the expression of hepatitis B surface antigen in yeast. pMA-56 was constructed from YRp7 (ref. 27) by (1) removing one of its *Eco*RI sites (digestion with *Eco*RI, fill-in by DNA polymerase I Klenow fragment and blunt-end ligation); (2) replacing the yeast *ars*-1 replication origin by a *Pst*I–*Eco*RI fragment containing the replication origin of yeast 2 μ plasmid; and (3) replacing the smallest *Eco*RI–*Bam*HI of the resulting plasmids by a 1,500-bp *Eco*RI–*Bam*HI fragment containing the yeast ADHI promoter[21]. Sixty microgrammes of pHBV-3200[13] were digested with *Eco*RI and *Hpa*I, and the 1,000-bp fragment containing the surface antigen gene was isolated and digested with *Tac*I; 10 μg of the resulting 800-bp fragment were ligated to *Eco*RI linkers and cloned in the *Eco*RI site of pBR322 (pHBS-5). The *Eco*RI fragment containing the HBsAg was isolated from pHBS-5 by preparative gel electrophoresis after digestion with *Eco*RI, and ligated to pMA-56 (previously linearized by *Eco*RI and treated with bacterial alkaline phosphatase). After transformation of *E. coli* cells (RR1) and isolation of DNA by a small scale procedure[28], recombinant plasmids were analysed by cleavage with restriction endonucleases. Plasmids in which the HBsAg gene was in line with the ADHI promoter (pHBS-16), and those with these regions in the opposite orientation (pHBS-20) were amplified and used to transform strain XV610-8C *a ade2 ade6 leu2 lys1 trp1 can1* as described elsewhere[19]. *b*, DNA sequence of the ADHI–HBsAg gene junction present in pHBS-16. The sequence was obtained by the method of Maxam and Gilbert[29]

Construction of plasmids and expression of HBsAg in yeast

The HBsAg-coding sequence used in these studies was isolated on a 835-bp *Taq*I–*Hpa*I fragment[13,16]. This fragment contains 26 bp preceding the AUG which encodes the N-terminal methionine of mature HBsAg. It lacks the DNA sequence of 489 or 522 bp that encodes a presumptive precursor which can be deduced from the viral genomic sequence[7]. The HBsAg gene fragment was joined to the yeast alcohol dehydrogenase (ADHI) promoter as described in Fig. 1 legend. The plasmid

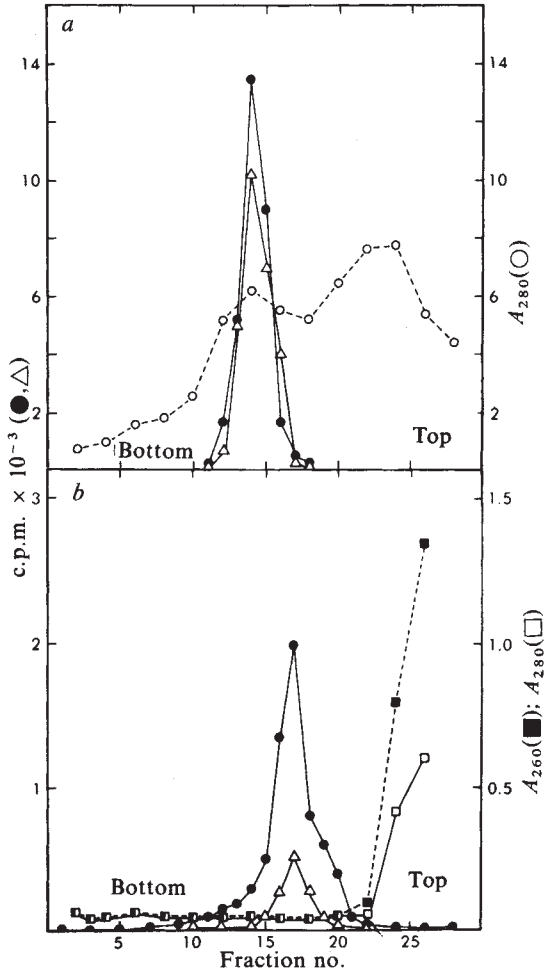

Fig. 2 *a*, CsCl gradient sedimentation of HBsAg from yeast extracts. yeast cells were grown in 0.67% yeast nitrogen base, 0.5% tryptophan-free casamino acids and 2% glucose. Cells from a 100 ml culture were pelleted, washed in 100 ml of buffer (10 mM phosphate *p*H 7.5, 0.1% Triton X-100), suspended in one volume of packed cells of the same buffer and broken by vortexing with glass beads (0.45–0.50 mm diameter). After centrifugation, a 0.5-ml aliquot of the extract was layered on to a 12 ml discontinuous 1.1–1.4 g cm^{-3} CsCl gradient in 10 mM phosphate buffer (*p*H 7.4) and run at 30,000 r.p.m. at 5 °C for 24 h in a beckman SW41 rotor. Fractions (0.5 ml) were collected from the bottom of the tube. The HBsAg activity was measured using the radioimmunoassay AUSRIA II (Abbott). The HBsAg used as standard was isolated from supernatants of PLC/PRF/5 cells[25,26]. *b*, Sucrose gradient sedimentation of HBsAg produced in yeast cells. Active fractions from the above CsCl gradient were pooled and dialysed against 10 mM phosphate buffer *p*H 7.5. A 0.5-ml aliquot was layered on to a 12 ml 5–30% sucrose gradient in 10 mM phosphate buffer containing 0.15 M NaCl and run at 33,000 r.p.m. at 5 °C for 6 h in a Beckman SW41 rotor. Fractions (0.5 ml) were collected and aliquots assayed as for *a*. A 0.5-ml sample of PCL/PRF/5 cell supernatant was used as standards in the same way. ●, Yeast; △, Alexander cells for *a* and *b*.

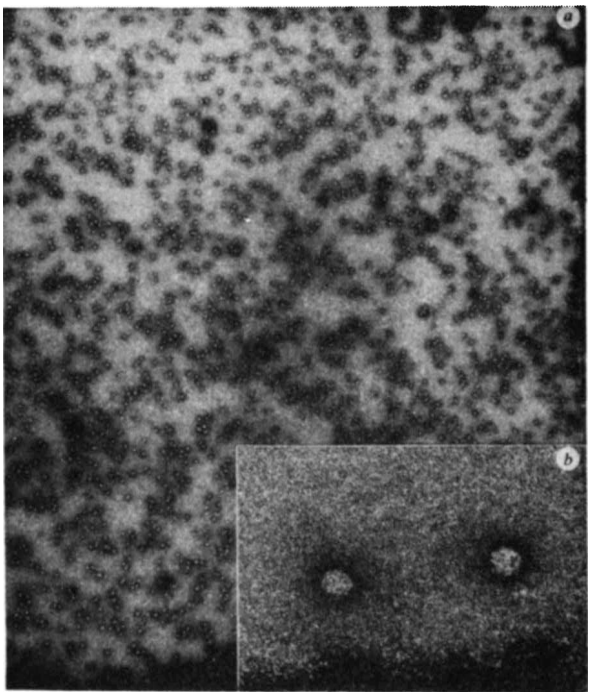

Fig. 3 Electron micrographs of HBsAg particles produced by yeast cells. *a*, Particles were purified by adsorption to a column of goat anti-HBsAg specific antibody covalently bound to Sepharose, eluted with 3 M potassium thiocyanate and visualized by negative staining with 2% phosphotungstic acid. ×25,000. *b*, Higher magnification of particles purified by a combination of CsCl and sucrose gradient sedimentation and visualized after negative staining with 2% uranyl acetate. ×250,000.

contains *Escherichia coli* vector pBR322, a replication origin derived from the yeast 2 μ plasmid, the *trp*1 gene for selection in yeast cells, and the ADHI promoter inserted into the *tet*^{r} region of pBR322. The efficacy of the ADHI promoter for expression of a linked coding sequence has recently been demonstrated by the production of interferon in this system[22]. Yeast cells (XV610-8C) were transformed by recombinant plasmids in which the HBsAg gene was placed either in the same 5′–3′ orientation as the ADHI promoter (pHBS-16) or in the opposite orientation (pHBS-20). Cell extracts from mid-log phase cultures of these strains were assayed for HBsAg by radioimmunoassay (AUSRIA II; Abbott). A substantial level of HBsAg was detected only in the cells transformed with pHBS-16; no surface antigen was observed in cells transformed with the plasmid in which the viral gene is incorrectly oriented. The amount of surface antigen protein made per 200 ml of yeast culture was ~2–5 μg, calculated from the radioimmunoassay data.

Nature of viral antigen produced in yeast

The predominant form of HBsAg produced by human cells is the so-called 22-nm particle. Its biophysical properties are well documented[23,24] and its immunological potency exceeds that of the pure protein[12]. To examine the form in which surface antigen is present in yeast, we subjected extracts to equilibrium sedimentation through a discontinuous CsCl gradient. A control tube containing HBsAg from a human hepatoma cell line (Alexander or PLC/PRF/5 cells[25,26]) was treated identically to provide a buoyant density marker. Surprisingly, HBsAg synthesized by yeast was found to band at the same density as that from the PLC/PRF/5 cell, that is, ~1.2 g cm^{-3} (Fig. 2*a*). Peak fractions of the CsCl gradient were analysed further by velocity sedimentation in sucrose gradients. Again the peak of HBsAg produced by yeast coincided exactly with HBsAg from human cells (Fig. 2*b*), which sediments at ~55S[24].

From the sedimentation data, it is apparent that HBsAg is synthesized in yeast in the form of particles or aggregates. The nature of these particles was further characterized by electron microscopy. HBsAg synthesized in yeast was purified again by a combination of sedimentation in CsCl and sucrose gradients. The immunoreactive material was adsorbed onto carbon film grids and negatively stained with uranyl acetate by Drs P. Bull and J. Garrido (Catholic University, Santiago, Chile). Alternatively, yeast HBsAg was purified by affinity chromatography and negatively stained with phosphotungstic acid by Drs G. Wampler, W. Ziegler, W. Miller and W. McAleer (Merck Sharp and Dohme Research Laboratories). When examined under the electron microscope, the yeast HBsAg preparation was found to contain particles (Fig. 3*a*,*b*) which appeared very similar to those secreted by Alexander cells. The particles purified and stained using the first method are ~20 nm in diameter. A more extensive analysis showed that the size of the purified particles eluting with 3 M ammonium thiocyanate from an affinity column is variable, the medium size being ~17 nm (W. Miller and W. McAleer, personal communication).

Preliminary tests have shown that these particles are antigenic, inducing antibodies in animal species (G. Buynak and W. McAleer, personal communication). It has been demonstrated previously[12] that integration of the HBsAg protein into a phospholipid structure to form the 22-nm particle dramatically increases its immunogenic properties. Thus the immunogenicity of the material synthesized in yeast is further evidence that its structure resembles that of the 22-nm particle.

The chemical composition of HBsAg molecules synthesized in yeast was determined by immunoprecipitation of ^{35}S-proteins labelled *in vivo* in a yeast strain transformed by the ADHI–HBsAg recombinant plasmid. SDS-gel electrophoresis of the anti-HBsAg immunoreactive material (Fig. 4) revealed a single band of apparent M_r 23,000, corresponding in size to the unglycosylated HBsAg component of PLC/PRF/5 cells[11]. No higher molecular weight bands could be detected, indicating that most of the molecules were not glycosylated in these conditions.

Conclusions

Expression of hepatitis B surface antigen coding sequences in yeast leads to the production of particles immunoreactive with anti-HBsAg antibodies. These particles are similar to those

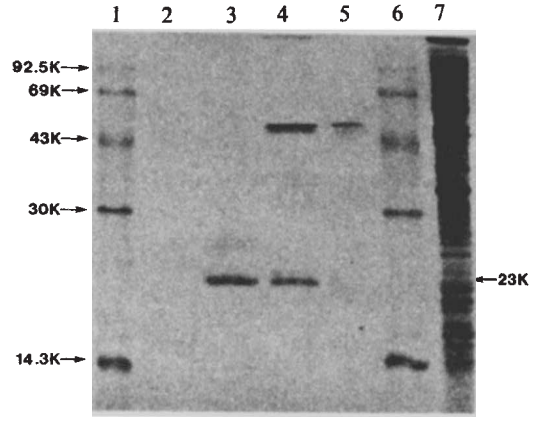

Fig. 4 Autoradiography of SDS-polyacrylamide gel of HBsAg proteins produced by yeast and PLC/PRF/5 cells. Yeast cultures and PLC/PRF/5 cells were grown in the presence of ^{35}S-methionine and ^{35}S-cysteine. Yeast cells were collected, broken by vortexing with glass beads and the resulting extracts clarified by centrifugation. Labelled HBsAg particles from PLC/PRF/5 cell supernants were concentrated by ultracentrifugation and used as a control. Proteins from yeast and PLC/PRF/5 cells were immunoprecipitated using the SAC technique[30]. Samples were run on 12.5% acrylamide gels according to Laemmli[31], subjected to fluorography[32], dried and exposed to X-ray film. Lanes 1 and 6, ^{14}C-methylated proteins as molecular weight standards: phosphorylase (92,500, 92.5K), bovine serum albumin (69K), ovalbumin (46K), carbonic anhydrase (30K) and lysozyme (14.3K); lane 2, PLC/PRF/5 cell supernatants plus normal human serum; lane 3, PLC/PRF/5 cell supernatant plus human anti-HBsAg serum; lane 4, yeast extracts from strain XV610-8C with pHBS-16 plus human anti-HBsAg serum; lane 5, yeast extracts from strain XV610-8C with pHBS-16 plus normal human serum; lane 7, aliquot of total yeast ^{35}S-labelled proteins. Numbers at the left refer to protein markers as above. The number at the right indicates the 23,000-M_r protein immunoprecipitated from yeast and PLC/PRF/5 cells.

made by human carrier patients or by a hepatoma cell line; they have identical sedimentation rates and buoyant density, suggesting a similar size and ratio of protein to lipid composition for yeast and for human particles. Electron microscopic observation established that the yeast particles are more variable in size and have a smaller mean diameter than the human particles. Furthermore, unlike the HBsAg from human cells, the yeast particle does not contain significant quantities of a higher molecular weight glycoprotein, indicating that glycosylation is required neither for the formation of the particulate structures nor for immunogenicity.

The construction we have used in the HBsAg-expressing plasmid eliminates that part of the gene encoding the leader peptide of putative pre-HBsAg[7]. Therefore, formation of particles in yeast implies that the polypeptide sequence of the surface antigen molecule contains the requisite instructions for assembly of apparently normal particles within the cytoplasmic milieu of the yeast cell. The leader region and other possible precursors formed by its translation are not required for the formation of this structure. Furthermore, no other hepatitis gene products or liver-specific cell products are necessary to form the particulate structure. It seems likely, therefore, that such particles can be formed in many other heterologous cell systems, perhaps even in bacteria. It remains to be determined where within the yeast cell the 22-nm particle is assembled, and what biochemical processes accompany it. An immediate advantage of the yeast system is that the physiological and genetic requirements for particle assembly may be systematically explored, making use of yeast mutants conditionally defective for the secretory pathway[18] and for other aspects of cellular metabolism.

The similarity in structure of the yeast particle to bona fide 22-nm particles and the high immunogenicity in animals emphasize the possible value of the HBsAg particle as a vaccine. The yeast HBsAg particles can in principle be produced in large quantities for this purpose. Further, the complete absence of intact HBV and/or human proteins in such preparations eliminates the possibility of secondary infections or autoimmunity problems as a result of the vaccine.

Finally, our findings illustrate the versatility of recombinant DNA methodology for the production of complex structures. Previously, recombinant DNA techniques as applied to microorganisms have only been used to produce simple proteins like insulin, growth hormone, interferon and enzymes. Our experiments suggest that more complicated structures can be formed in alternative hosts like yeast provided the genes for the requisite organizing proteins are introduced and efficiently expressed. This offers an effective approach for the analysis of organelles in higher organisms.

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Note added in proof: Since the submission of this paper, particles containing a different HBV protein, the virus core antigen, have been reported in *E. coli* carrying a recombinant plasmid[33].

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1. Vyas, G. N., Cohen, S. N. & Schmid, R. (eds) *Viral Hepatitis. A Contemporary Assessment of Etiology, Epidemiology, Pathogenesis and Prevention* (Franklin Institute Press, Philadelphia, 1978).
2. Zuckerman, A. J. *J. Tox. envir. Hlth* **5**, 275–280 (1979).
3. Prince, A. M. *Hepatology* **1**, 73 (1981).
4. Summers, J., O'Connell, A. & Millman, I. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4597–4601 (1975).
5. Pasek, M. *et al. Nature* **282**, 575–579 (1979).
6. Galibert, F., Mandart, E., Fitoussi, F., Tiollais, P. & Charnay, P. *Nature* **281**, 646–650 (1979).
7. Valenzuela, P., Quiroga, M., Zaldivar, J., Gray, P. & Rutter, W. J. in *Animal Virus Genetics* (eds Fields, B. N., Jaenisch, R. & Fox, C. F.) 55–77 (Academic, New York, 1980).
8. Robinson, W. S. & Greenman, R. C. *J. Virol.* **13**, 1231–1236 (1974).
9. Hruska, J. F. & Robinson, W. S. *J. med. Virol.* **1**, 119–124 (1977).
10. Shih, J. W. K. & Gerin, J. L. *J. Virol.* **21**, 1219–1222 (1977).
11. Peterson, D. L. *J. biol. Chem.* **256**, 6975–6983 (1981).
12. Cabral, G. A. *et al. J. gen. Virol.* **38**, 339–350 (1978).
13. Valenzuela, P. *et al. Nature* **280**, 815–819 (1979).
14. Burrell, C. J., Mackay, P., Greenway, P. J., Hofschneider, P. H. & Murray, K. *Nature* **279**, 43–47 (1979).
15. Sninsky, J. J., Siddiqui, A., Robinson, W. S. & Cohen, S. N. *Nature* **279**, 346–348 (1979).
16. Edman, J. C., Hallewell, R. A., Valenzuela, P., Goodman, H. M. & Rutter, W. J. *Nature* **291**, 503–506 (1981).
17. Mackay, P. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 4510–4514 (1981).
18. Novick, P., Field, C. & Schekman, R. *Cell* **21**, 205–225 (1980).
19. Beggs, G. D. *Nature* **275**, 104–109 (1978).
20. Petes, P. D. *A. Rev. Biochem.* **49**, 845–876 (1980).
21. Ammerer, G., Hitzeman, R. A., Hagie, F. E., Barta, A. & Hall, B. D. *Recombinant DNA, Proc. 3rd Cleveland Symp. Macromolecules* (ed. Walton, A. G.) 185–197 (Elsevier, Amsterdam, 1981).
22. Hitzeman, R. A. *et al. Nature* **293**, 717–722 (1981).
23. Gerin, J. L., Purcell, R. H., Hoggan, M. D., Holland, P. V. & Chanock, R. M. *J. Virol.* **4**, 763–768 (1969).
24. Gerin, J. L., Holland, P. V. & Purcell, R. H. *J. Virol.* **7**, 569–576 (1971).
25. Macnab, G. M., Alexander, J. J., Letcasas, G., Bey, E. M. & Urbanowicz, J. M. *Br. J. Cancer* **34**, 509–515 (1976).
26. Alexander, J. J., Bey, E. M., Geddes, E. W. & Letcasas, G. *S. Afr. med. J.* **50**, 2124–2128 (1976).
27. Tschumper, G. & Carbon, J. *Gene* **10**, 157–166 (1980).
28. Birnboim, H. C. & Doly, J. J. *Nucleic Acids Res.* **7**, 1513–1523 (1979).
29. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65**, 490–565 (1980).
30. Martial, J. A., Hallewell, R. A., Baxter, J. D. & Goodman, H. M. *Science* **205**, 602–607 (1979).
31. Laemmli, U. K. *Nature* **227**, 680–685 (1977).
32. Bonner, W. M. & Laskey, R. A. *Eur. J. Biochem.* **46**, 83–88 (1974).
33. Cohen, B. J. & Richmond, J. E. *Nature* **296**, 677–678 (1982).

LETTERS TO NATURE

Evidence for an oblique magnetic solar rotator

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The observation by Claverie *et al*[1]. of fine structure in the peaks in the power spectrum of low-degree 5-min solar oscillations has been interpreted as being a result of rotational splitting. Claverie *et al.*[1] claim that their measurements imply that an appropriately weighted average $\bar{\Omega}$ of the interior angular velocity $\Omega(r)$ of the Sun is about twice the value of Ω at the surface. At first sight their claim looks doubtful, because all $2l+1$ components of the set of modes of degree l appear in the spectrum, whereas only $l+1$ of them should be detectable. However, Isaak[2] has recently speculated that the additional components are produced by an intense rotating magnetic core, such as had been postulated by Dicke[3],[4] to account for the 12.2-day periodic component in the Princeton oblateness data[5–9]. Isaak[2] pointed out that the mean period $2\pi/\Omega \simeq 15$ days of the solar interior that was inferred from the Birmingham data[1] is consistent with a central core rotating with the 12.2-day period and an outer envelope rotating with the photosphere. Moreover, his rough estimate of a few megagauss for the r.m.s. magnetic field that is required to support his conjecture is within about a factor 10 of that required by Dicke[3]. Thence he concluded that the Birmingham data[1] provide the first clear empirical evidence for an intense internal solar magnetic field. Here I examine this evidence in more detail, and show that the conclusion is premature. If the magnetic core does exist, the 5-min oscillations provide no clear evidence that it is rotating rapidly. Furthermore, unless one accepts a contrived magnetic-field configuration, an explanation for the $2l+1$ components of the multiplets is still lacking.

With respect to spherical polar coordinates (r, θ, ϕ) about any axis, the linear eigenfunctions of oscillation of a spherically symmetrical star of radius R are separable in radial and angular coordinates. For example, the radial component δr of the dis-

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