parent. The magnitudes of the signals from frozen and lyophilized samples are initially about the same, but as the tumors develop the number of spins decreases in the frozen samples and remains the same in the lyophilized ones.

Key unanswered questions include (i) the nature of the signal from lyophilized samples; (ii) the nature of the signal from frozen samples; (iii) the relationship between these signals; (iv) whether the signals observed when a tumor is present are qualitatively different from those observed in normal tissues; and most important (v) whether these signals have any practical or theoretical significance in cancer.

It should be possible to obtain definitive answers to at least some of these questions. For example, the changes in intensity, line shape, and line width which we observed indicate that the signal from lyophilized samples is different from that from frozen nonlyophilized samples. There are similar indications that the signals from tumor samples are not identical to those from controls. The signal-to-noise ratios for these samples are probably sufficient to permit more detailed ESR studies.

Admission of oxygen to lyophilized samples provides an additional means of enhancing the signal-to-noise ratios (*11*) and of determining differences between different samples. With the introduction of oxygen, qualitative effects on the line shape, effects on signal intensity, and the time pattern of these changes could be studied.

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References and Notes

1. H. Swartz, *Adv. Cancer Res.* **15**, 227 (1972).
2. A. Saprin, Ye. Minenkova, L. Nagler, Yu. Kaznacheyev, S. Kruglyak, K. Kruglyakova, N. Emanuel, *Biofizika* **12**, 1099 (1967).
3. A. Saprin, L. Nagler, Ye. Koperina, K. Kruglyakova, N. Emanuel, *ibid.* **11**, 706 (1966); A. Saprin, E. Klochko, V. Chibrikin, K. Kruglyakova, N. Emanuel, *ibid.*, p. 443; A. Saprin, Ye. Minenkova, L. Nagler, Ye. Koperina, S. Kruglyak, K. Kruglyakova, Ye. Vermel, N. Emanuel, *ibid.*, p. 616; A. Saprin, Ye. Minenkova, L. Nagler, S. Kruglyak, K. Kruglyakova, N. Emanuel, *ibid.* **12**, 1022 (1967); A. Saprin, E. Klochko, K. Kruglyakova, V. Chilbrikin, N. Emanuel, *Dokl. Akad. Nauk SSSR* **167**, 222 (1965); D. Driscoll, C. Dettmer, J. Wallace, A. Neaves, *Curr. Mod. Biol.* **1**, 275 (1967); D. Driscoll, J. Wallace, C. Dettmer, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **26**, 626 (1967); J. Wallace, *Crit. Rev. Radiol. Sci.* **3**, 89 (1972).
4. H. Swartz, C. Mailer, S. Ambegaonkar, W. Antholine, D. McNellis, S. Schneller, *Cancer Res.* **33**, 2588 (1973); J. Duchesne, R. Goutier, R. Maréchal, A. Van de Vorst, *Phys. Med. Biol.* **20**, 305 (1975); A. Vithayathil, J. Ternberg, B. Commoner, *Nature (London)* **207**, 1246 (1965); H. Abe and Y. Kurata, *Gann* **65**, 75 (1974); J. Mallard and M. Kent, *Nature (London)* **210**, 588 (1966); N. Dodd and J. Giron-Conland, *Br. J. Cancer* **28**, 257 (1973).
5. More details on the lyophilization method will appear elsewhere (P. Gutierrez and H. Swartz, in preparation).
6. The tumor line was kindly supplied by the WARF (Wisconsin Alumni Research Foundation) Institute, Madison, Wis.
7. H. Swartz and R. P. Molenda, *Science* **148**, 94 (1965).
8. T. Maruyama *et al.*, *Cancer Res.* **31**, 179 (1971).
9. A. Vanin, L. Vaknina, A. Chetverikov, *Biofizika* **15**, 1044 (1970).
10. R. Andrew, *Phys. Rev.* **91**, 425 (1953).
11. R. Heckly, in *Biological Applications of Electron Spin Resonance*, H. Swartz, J. Bolton, D. Borg, Eds. (Wiley, New York, 1972), p. 211.
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Intergeneric Transfer of Genes Involved in the *Rhizobium*-Legume Symbiosis

Abstract. *Genes that seem to be involved in the initial steps of infection of a legume by* Rhizobium *have been transferred, by transformation, to mutant strains of* Azotobacter vinelandii *that are unable to fix nitrogen. These genes code for a surface antigen that binds specifically to a protein from the host plant.*

Bacteria of the genus *Rhizobium* are capable of forming a complex nitrogen-fixing symbiotic association with leguminous plants. Bacteria of the genus *Azotobacter*, on the other hand, fix nitrogen without a requirement for a symbiotic host. Intergeneric transformation between *Rhizobium* and *Azotobacter* was first described by Sen *et al.* (*1*). *Azotobacter* transformants were selected for resistance to crystal violet or streptomycin. Some of these transformants resembled the *Rhizobium* donor strains in several nonselected biochemical properties. More recently, Venkataraman *et al.* (*2*) transformed *R. trifolii* with DNA from *A. chroococcum* and obtained transformants that grew on a nitrogen-free medium and, in the free-living state, reduced acetylene to ethylene. By utilizing DNA from several strains of *Rhizobium*, Page (*3*) was able to transform mutant strains of *A. vinelandii* which are unable to fix nitrogen (Nif^- strains) to Nif^+ strains. We thought that it might be possible to transfer, to *A. vinelandii*, genes of *R. trifolii* that are specifically required for root hair infection of *Trifolium repens* (white clover).

Trifoliin is a protein from clover that seems to be necessary for the initial stages of the infection process by *R. trifolii* (*4*, *5*). There is increasing evidence that lectins, such as trifoliin, are involved in the specificity of a *Rhizobium* species for its natural leguminous host plant (*5*–*7*). Immunochemical studies indicated that infective *R. trifolii* cells and clover roots have unique cross-reactive surface antigens (*7*). Preferential adsorption of infective *R. trifolii* to root hairs of clover may result from cross bridging of the cross-reactive surface antigens by trifoliin (*7*).

We transformed (*8*) three Nif^- strains of *A. vinelandii* to Nif^+ strains with crude DNA preparations from *R. trifolii* strain 0403 (obtained from P. S. Nutman). The transformation frequencies ranged from 1.5×10^{-7} to 2.4×10^{-6} (Table 1). Forty-six Nif^+ transformants from a cross with strain UW10 as the recipient were studied further. These transformants produced a green diffusible pigment characteristic of the recipient strain. The recipient and transformant cells appeared identical to each other and distinctly different from donor cells in both size and morphology when examined by phase-contrast microscopy. Three azotophages that were specific for the recipient strain formed

Table 1. Intergeneric transformation with *R. trifolii* as the donor and *A. vinelandii* as recipient. Transformation was conducted as described by Page and Sadoff (*8*), except that 2 percent sucrose replaced glucose in the transformation medium and the donor strain was lysed in 0.10 percent sodium dodecyl sulfate. The donor strain, *R. trifolii*, was cultured in a defined medium (*12*) with 1 percent D-mannitol as the carbon source. Growth and phenotypes of the *A. vinelandii* Nif^- recipient strains have been described (*13*).

Recipient strains	Nif phenotype*	Reversion to Nif^+†	Nif^+ transformation frequency‡
UW1	I^-II^-	$<1.1 \times 10^{-8}$	1.5×10^{-7}
UW6	I^-II^+	$<6.3 \times 10^{-8}$	2.6×10^{-7}
UW10	I^-II^+	$<2.8 \times 10^{-8}$	2.4×10^{-6}

*I and II represent the Mo-Fe and Fe components of nitrogenase, respectively. The superscripts + or − refer to the presence or absence of activity. †The frequency of reversion was based on the number of cells plated. ‡The Nif^+ transformation frequencies were calculated as the number of Nif^+ transformants per milliliter divided by the total number of cells per milliliter.

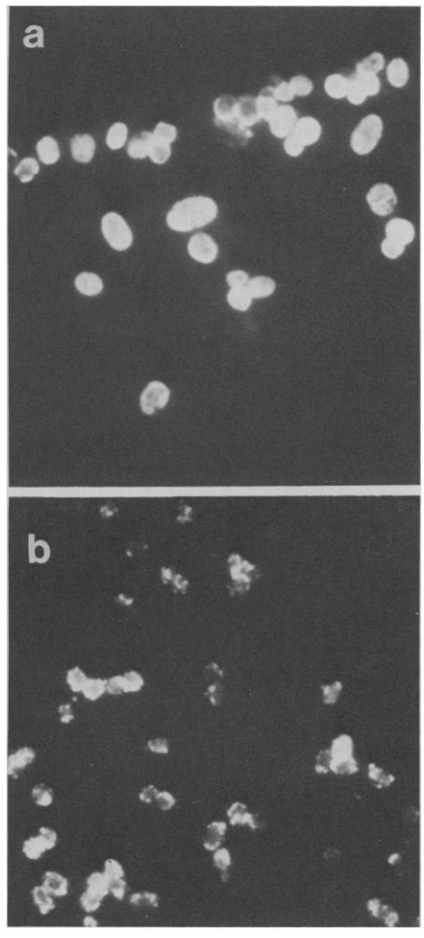

Fig. 1. Immunofluorescence of the hybrid strains RtAv10-54 (a) and RtAv10-29 (b) after treatment with rabbit antiserum to clover root antigens and fluorescein isothiocyanate conjugated goat antiserum to rabbit immunoglobulins. Fluorescence microscopy was performed as previously described.

plaques on the hybrid transformants. The transformants reduced acetylene to ethylene and grew aerobically in nitrogen-free medium with a generation time of 6.8 hours. Thus, it is apparent that the transformants have retained many of the characteristic features of the recipient strains and therefore were not contaminants.

Dazzo *et al* (*9*) indicated that trifoliin specifically agglutinates *R. trifolii* but not other species of *Rhizobium*. A tube agglutination assay was used to screen the Nif$^+$ transformants for the presence of trifoliin-binding sites. The results indicated that 13 percent of the Nif$^+$ transformants were agglutinated by trifoliin (Table 2). The transformants that were agglutinated by trifoliin were also agglutinated by rabbit antiserum prepared against clover root antigens (Table 2). The antiserum to clover root antigens failed to agglutinate the remaining transformants. This result indicated that transformants which were agglutinated by trifoliin also contained a cell-surface antigen that is cross-reactive with a component of clover root wall. Since all of the transformants that have trifoliin-binding sites are also agglutinated by antiserum to clover root antigens, it is possible that the trifoliin-binding site and the cross-reactive antigen constitute the same cell-surface component, as was suggested by the lectin cross-bridging hypothesis (*7*).

In order to determine whether the agglutination reactions are specific for *Azotobacter* cells transformed with DNA from *R. trifolii*, we also performed the agglutination tests on *Azotobacter* transformed with DNA from *R. japonicum*, which has *Glycine Max* (soybean) as its normal host. Nif$^+$ transformants from a cross with DNA from *R. japonicum* strain 61A76 (obtained from J. C. Burton) and *A. vinelandii* strain UW10 were tested for agglutination by trifoliin and antiserum to clover root antigens. No agglutination reaction was observed with ten Nif$^+$ transformants (Table 2). Four of these ten transformants had been observed to be agglutinated by rabbit antiserum prepared against whole cells of *R. japonicum* (unpublished data). Strain UW10 and a Nif$^+$ revertant of strain UW10 served as negative controls. Thus the agglutination reactions of the transformants appear to be specifically determined by *Rhizobium* donor species. None of the transformants that were agglutinated by trifoliin were able to nodulate white clover roots.

Indirect immunofluorescence (*7*) was used to confirm the specific cell surface binding of antiserum to clover root antigens. Figure 1a shows that the hybrid strain RtAv10-54 binds antiserum to clover root antigens uniformly over its cell surface, whereas strain RtAv10-29 (Fig. 1b) appears to bind the antibody in a nonuniform patchy fashion. Results with wild-type *A. vinelandii*, strain UW10, and a Nif$^+$ revertant of strain UW10 were negative. Further studies with the hybrid strains may help in understanding the regulation of the biosynthesis and compartmentation of the cross-reactive antigen on the surfaces of these cells.

A backcross between the hybrid transformant, RtAv10-54, and *A. vinelandii* strain UW10 was performed to determine whether the Nif$^+$ phenotype and the ability to bind trifoliin are transferred together. Only two of the ten Nif$^+$ transformants examined from such a cross were agglutinated by trifoliin. Therefore, it appears that the genetic loci responsible for the Nif$^+$ phenotype and ability to bind trifoliin are not closely linked.

Table 2. Agglutination of hybrid Nif$^+$ transformants with trifoliin and antiserum to clover root antigens. The cross consisted of the donor as indicated and *A. vinelandii* UW10 as the recipient. Tube agglutination reactions were conducted as described (*5*). The transformants were grown for 2 days at 30°C on modified Burk's agar medium (*14*) supplemented with 400 μg of nitrogen per milliliter as ammonium acetate. The trifoliin agglutination reaction mixtures contained 0.25 m*M* $MgSO_4$, 0.5 m*M* $CaCl_2$, and 0.15 m*M* $MnCl_2$.

Donor	Transformants (No.)		
	Tested	Agglutinated by	
		Trifoliin*	Antiserum†
R. trifolii	46	6	6‡
R. japonicum	10	0	0

*Trifoliin (30 μg/ml) was shown to be pure by acrylamide gel electrophoresis. †Antiserum to clover root antigens was prepared as described (*7*). ‡These six strains were the same as the ones that gave a positive reaction to trifoliin.

Plasmid DNA molecules of unknown function have been detected (*10*) by physical techniques in several strains of *Rhizobium*. To determine whether transfer of the Nif$^+$ and trifoliin-binding phenotypes from *R. trifolii* to *A. vinelandii* could be correlated with plasmid transfer, DNA preparations (*11*) from *R. trifolii* and from several of the hybrid transformants were centrifuged (*11*) to equilibrium in CsCl gradients in a Spinco model E analytical ultracentrifuge. *Micrococcus lysodeikticus* DNA was used as a density standard. Examination of *R. trifolii* DNA revealed a chromosome band (buoyant density, 1.721 g/ml) and a satellite band (buoyant density, 1.718 g/ml) that was approximately 5 to 10 percent of the size of the chromosome band. DNA preparations from wild-type *A. vinelandii*, from strain UW10, and from four hybrid transformants (three of which had previously been shown to bind trifoliin and antiserum to clover root antigen) showed only a chromosomal DNA band (buoyant density, 1.723 g/ml). Thus, it appears that the *R. trifolii* satellite DNA was either not transferred to *A. vinelandii* or was not maintained in the hybrid transformants in the form of a stable extrachromosomal element.

Thus, intergeneric transformation between *Rhizobium* and *Azotobacter* may facilitate detailed studies of *Rhizobium* genes involved with the nitrogen-fixing symbiosis in leguminous plants.

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References and Notes

1. M. Sen, T. K. Pal, S. P. Sen, *Antonie van Leeuwenhoek J. Microbiol. Serol.* **35**, 533 (1969).
2. G. S. Venkataraman, P. Roychaudhury, L. E. Henriksson, E. Henriksson, *Curr. Sci.* **44**, 520 (1975).
3. W. J. Page. *Abstr. Annu. Meet. Am. Soc. Microbiol.* **77**, 199 (1977).
4. F. B. Dazzo, in *Cell Wall Biochemistry Related to Specificity in Host-Plant Pathogen Interactions*, B. Solheim and J. Raa, Eds. (Columbia Univ. Press, New York, 1977), p. 389.
5. F. B. Dazzo and W. J. Brill. *Appl. Environ. Microbiol.* **33**, 132 (1977).
6. B. B. Bohlool and E. L. Schmidt, *Science* **185**, 269 (1974); J. S. Wolpert and P. Albersheim, *Biochem. Biophys. Res. Commun.* **70**, 729 (1976).
7. F. B. Dazzo and D. H. Hubbell, *Appl. Microbiol.* **30**, 1017 (1975).
8. W. J. Page and H. L. Sadoff, *J. Bacteriol.* **125**, 1080 (1976).
9. F. B. Dazzo, W. E. Yanke, W. J. Brill, *Biochim. Biophys. Acta*, in press.
10. G. Tshitenge, N. Luyindula, P. F. Lurquin, L. Ledoux, *ibid.* **414**, 347 (1975); W. D. Sutton, *ibid.* **366**, 1 (1974); W. Zurkowski and Z. Lorkiewicz, *J. Bacteriol.* **128**, 481 (1976).
11. D. Perlman and R. H. Rownd, *J. Bacteriol.* **123**, 1013 (1975).
12. P. E. Bishop, J. G. Guevara, J. A. Engelke, J. J. Evans, *Plant Physiol.* **57**, 542 (1976).
13. V. K. Shah, L. C. Davis, J. K. Gordon, W. H. Orme-Johnson, W. J. Brill, *Biochim. Biophys. Acta* **292**, 246 (1973).
14. G. W. Strandberg and P. W. Wilson, *Can. J. Microbiol.* **14**, 25 (1977).

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Mapping the Locus of the *H-Y* Gene on the Human Y Chromosome

Abstract. *The* H-Y *locus is on the short arm of the human Y chromosome in most individuals but on the long arm in at least one of 17 individuals with structural abnormalities of the Y.*

When female mice are sensitized with skin grafts or lymphoid cells from males of the same inbred strain, they produce antibody which identifies a plasma membrane component called H-Y (histocompatibility-Y) antigen (*1*). Recently, we have shown that the gene governing expression of H-Y antigen is widespread among vertebrates, occurring in several divergent species including man (*2*). In all species so far examined, H-Y antigen is associated with the heterogametic (XY) sex. H-Y antigen is not present in XO female mice but is present in XXY male mice (*3*) and in *Tfm* mice, that is, XY intersexual mice with testicular feminization (*4*). These findings support the hypothesis that a gene on the Y chromosome is necessary for the expression of H-Y antigen and that androgen responsiveness, which is absent in testicular feminization, is not required. A structural or regulatory gene for H-Y antigen expression is known to be located on the Y chromosome of man; white blood cells from human males with two Y chromosomes express more H-Y antigen than cells from normal XY males (*5*). However, the exact position of the human *H-Y* gene remains to be determined.

In view of the possible role of H-Y antigen in the differentiation of the mammalian testis (*6*), precise localization of the Y-linked *H-Y* gene may prove crucially important (i) in the detection of virilized gonads in phenotypic females and (ii) in understanding the etiology of intersexual phenotypes associated with structural abnormalities of the Y chromosome. Therefore as a first step in localizing the *H-Y* gene, we have studied H-Y antigen expression in patients exhibiting deletions or other structural modifications of the Y. A note on the findings in a few of these patients has been published (*7*).

Serological detection of H-Y antigen was based on the ability of white blood cells (WBC) to absorb H-Y antibody. H-Y antiserums were collected from inbred female mice that had been sensitized with serial inoculations of male spleen cells. Before reaction with mouse sperm, H-Y antiserms were pooled and subdivided, and portions were absorbed with WBC from normal human males (46,XY), normal human females (46,XX), and patients (for example, male, 46,XYq-). Positive absorption (indicating the presence of H-Y antigen on the absorbing WBC) was manifested as a decrease in the reaction of H-Y antiserums with target sperm. Thus WBC from human males (H-Y$^+$) specifically absorbed (bound) H-Y antibodies from H-Y antiserums and thereby decreased the ability of these serums to react with mouse sperm in both the sperm cytotoxicity test (*5*) and the mixed hemadsorption hybrid antibody test (*8*).

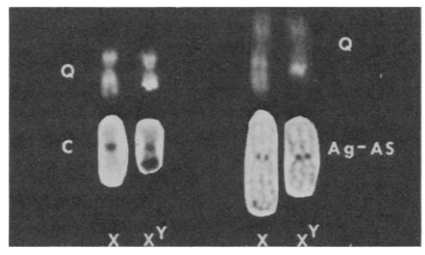

Fig. 1. The sex chromosomes from two cells of case 8. Those on the left were photographed first to show quinacrine banding (*Q*) and then to show C banding (*C*). Those on the right were photographed first to show Q banding and then to show silver-staining (*Ag-AS*).

Of the 17 individuals tested, 15 were H-Y antigen positive (Table 1). The correlations of H-Y antigen expression with karyotype, sexual phenotype, and appearance of the gonads are summarized in Table 1.

The *H-Y* locus is not on the intensely quinacrine fluorescent distal region of the Y chromosome. H-Y antigen was present in cases 1 to 4 and 9 to 16 despite the absence of this region in all 12 of these cases. Case 1 had a deletion of approximately the distal half of the Y, including almost all of the quinacrine-bright and 5-methylcytosine-rich materials. Cases 2 to 4 had Y-derived chromosomes of average size and typical morphology, but in each case the distal portion failed to show intense fluorescence after quinacrine staining, and no portion of the Y showed the intense binding of antiserum to 5-methylcytosine that is characteristic of normal Y chromosomes (*9*). On the other hand, in case 5 the quinacrine-bright portion of the Y was present, but the absence of H-Y antigen confirmed the exclusion of this region as the locus of the *H-Y* gene.

Evidence that the *H-Y* locus may be on the short arm of the Y chromosome was provided by several cases. In the first of these, case 5, the abnormal Y chromosome was symmetrical and had the characteristics of an isochromosome of the long arm of the Y (*10*); that is, only the short arm was absent. Since H-Y antigen was also absent from this female, who had Turner syndrome and streak gonads, the *H-Y* locus must have been on the short arm of the Y. Case 6 also had Turner syndrome (although the gonads were not examined) and was H-Y antigen-positive with no evidence of the increased amount of antigen seen in individuals with two Y chromosomes (*5*). This patient, a female, had a slightly asymmetrical Y chromosome with duplicated long arms, which was not an isochromosome because there was additional material on one arm forming a quinacrine-dull band adjacent to the centromere. The simplest explanation for these results is that the extra band represents material from the short arm of the Y and that it contains the *H-Y* locus.

The location of the *H-Y* locus on the short arm of the Y is also indicated in case 7. This male had testes and was