situ crystallization in a closed system. Cumulate compositions are sometimes used to estimate coexisting liquids; but the cumulate composition may be far removed from erupted compositions. Substantial variations in incompatible trace elements for constant major elements have been used as evidence of variations in the extent of partial melting; but this is a general characteristic of the *in situ* process. As the chemical consequences of *in situ* crystallization become better understood, it will be necessary to develop tests to determine how many of these features of igneous petrogenesis it actually does account for. It is possible that the thermal conditions and density relationships for different tectonic settings could cause fundamentally different evolutionary paths as a result of the nature of return of residual liquid from the solidification zone.

If further work confirms the importance of the process, subsequent efforts will need to take account of the vertical zonation of many magma chambers (see, for example, refs 12, 36–40), the variations in liquid compositions in the solidification zone, interaction with pre-existing crystals as the liquid moves through the solidification zone, and kinetic effects. Although this paper has focused on magma chambers, it is possible that a similar process may lead to complex changes in liquid composition as a magma moves through a conduit system with a partially molten solidification zone. □

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Bacterial conjugative plasmids mobilize DNA transfer between bacteria and yeast

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Conjugative plasmids of *Escherichia coli* can mobilize DNA transmission from this bacterium to the yeast *Saccharomyces cerevisiae*. The process shares some of the features of conjugation between bacteria and could be evolutionarily significant in promoting trans-kingdom genetic exchange.

TRANSMISSION of genetic information between distant taxonomic groups could create organisms with unique evolutionary potential. One well-studied example of such gene transmission occurs between the bacterium *Agrobacterium tumefaciens* and certain plant species (for review, see ref. 1). It seems that DNA transfer from *A. tumefaciens* to plants is mechanistically similar to bacterial conjugation[2,3]. Most strikingly, conjugative plasmids can substitute for tumour-inducing plasmid functions necessary for transmission of DNA to plants, provided that certain tumorigenic-encoded virulence functions that contribute to DNA transfer are also present[4]. Here we investigate whether bacterial conjugation could provide a general mechanism for inter-kingdom genetic exchange using derivatives of two classes of bacterial conjugative plasmids: broad-host-range plasmids, such as R751, which promote conjugation between a large number of bacterial species, including between Gram-negative and Gram-positive species[5–7], and the F plasmid (fertility factor), which confers a limited-host-range phenotype, its conjugation being limited to *E. coli* and related Enterobacteriaceae[8]. Here we show that both broad-host-range and limited-host-range conjugation plasmids can transmit DNA from bacteria to yeast.

Transfer of plasmids between bacteria by conjugation requires cell contact and DNA metabolism[9–12]. Transmission to the new host also requires that the plasmid should replicate, either autonomously or by association with a host replicon. The donor cell in a conjugal relationship contains a self-transmissible plasmid which encodes the capacity to carry out the transfer functions. The plasmid *tra* locus encodes cell-surface components as well as mobilization proteins (*mob* gene products) which promote DNA transfer by interaction with a *cis*-acting sequence termed the origin of transfer (*oriT*). We use representatives of three different plasmid groups: R751, ColE1 and the fertility factor F. For simplicity, suffixes are used to denote the origin

of conjugation functions: for example, *mob*-R and *oriT*-R refer to the *mob* and *oriT* functions of the R751 group respectively (Table 1).

DNA transmission from bacteria to yeast

To determine whether plasmids can be transmitted from bacteria (*E. coli*) to yeast (*S. cerevisiae*) by conjugation, we have used two bacterial strains, one containing both a self-transmissible plasmid and a plasmid that can replicate and be selected in yeast, and the other containing only the yeast plasmid. The plasmid pDPT51, a derivative of the broad-host-range plasmid R751, contains *mob*-R and *oriT*-R for self-transmission and also the *mob*-C region from ColE1 (Table 1). Thus, pDPT51 can mobilize *in trans* other plasmids, such as pBR322, that contain the *oriT* region from ColE1 (*oriT*-C, or the *bom* site[9]). We used the yeast plasmid YEp13 (Table 1), which is a yeast-bacteria shuttle vector derived from pBR322. YEp13 carries the yeast *LEU2* gene and a segment of the yeast 2μ plasmid, allowing selection and replication of the plasmid in yeast.

We tested *E. coli* cells containing both plasmids for the ability to transmit plasmid DNA to yeast cells and to other *E. coli* strains using a standard protocol for bacterial conjugation (see Table 2 legend). When donor bacteria are mixed with *leu2*$^-$ yeast cells, Leu$^+$ yeast are detected at a frequency of 3×10^{-7} Leu$^+$ cells per recipient (Table 2*a*). We confirmed that the Leu$^+$ yeast cells contained YEp13 by restriction-endonuclease analysis of plasmid DNA recovered from these cells. Although we presume that pDPT51 was also transferred to yeast, we did not detect its transmission because it lacks a replication sequence for propagation in yeast.

The frequency of transmission of YEp13 ranges from 3×10^{-8} to 5×10^{-5} Leu$^+$ cells per recipient and can be as high as 7×10^{-2} Leu$^+$ cells per donor, depending partly on the ratio of donor to recipient cells in the mating assay (Tables 2 and 3). In bacterial conjugation experiments, transconjugant bacteria arose at a frequency of $\sim3\times10^{-1}$ transconjugants per recipient (Table 2). Initially we made no attempt to optimize the ratio of bacteria to yeast in the mating assay, but later found that an excess of bacterial cells reduced the frequency of DNA transmission, which has been found for conjugation between bacteria[9]. The donor:recipient ratios used in each experiment are given in Tables 2 and 3.

To see whether the appearance of Leu$^+$ yeast cells was dependent on the conjugative plasmid, *E. coli* carrying only YEp13 was mixed with yeast. No Leu$^+$ yeast was observed ($<1\times10^{-9}$ Leu$^+$ cells per recipient; Table 2*a*), indicating that DNA transmission was dependent on a conjugation plasmid. We therefore

TABLE 1 Plasmid functions

Plasmid	Conjugation functions	Selectable markers* in bacteria	in yeast	Refs
R751	*tra*-R, *mob*-R, *oriT*-R	Tp^r	none	(19)
pDPT51	*tra*-R, *mob*-R, *oriT*-R, *mob*-C	Tp^r, Am^r	none	(20)
pDPT51:LEU2	*tra*-R, *mob*-R, *oriT*-R, *mob*-C	Tp^r, Am^r	*LEU2*	This work
YEp13	*oriT*-C	Tc^r, Am^r	*LEU2*	(21)
YEp24	*oriT*-C	Tc^r, Am^r	*URA3*	(22)
pAL2	none	Cm^r	*LEU2*	This work
pACYC184	none	Cm^r, Tc^r	none	(15)
pFLEU2	*tra*-F, *mob*-F, *oriT*-F	Am^r	*LEU2*	This work
pRS2405	*tra*-F, *mob*-F, *oriT*-F	Am^r	none	(23)

Plasmids R751, pDPT51, YEp13, YEp24, pACYC184, and pRS2405 have been described (refs 15, 19–23). Plasmids R751 and pDPT51 are broad-host-range conjugative plasmids of the P incompatibility group. Plasmid pRS2405 is a limited-host-range plasmid of the F incompatibility group. pAL2 was constructed by cloning an *Xho*I–*Hin*dIII fragment that contains *LEU2* and 2μ sequences from YEp13 into the *Sal*I and *Hin*dIII sites of pACYC184, resulting in the deletion of the *tet*^{r} gene of pACYC184. pFLEU2 and pDPT51:*LEU2* were constructed in multiple steps. First, the *Xho*I–*Hin*dIII 2μ, *LEU2* fragment of YEp13 was cloned into the *Sal*I–*Hin*dIII sites of pUC12 (ref. 24). The 2μ, *LEU2* fragment was then cloned as an *Eco*RI (from the pUC12 polylinker)–*Hin*dIII fragment into the *Eco*RI and *Hin*dIII sites of pBR322 (ref. 25). In the resulting plasmid, the 2μ, *LEU2* segment is flanked by *Bam*HI sites, one from the pUC polylinker and one in the *tet*^{r} gene of pBR322. To create pFLEU2, the *Bam*HI fragment was inserted into the *Bam*HI site of pRSF2405. To create pDPT51:*LEU2*, the *Bam*HI fragment was first cloned into the *Bam*HI site of pNK1207 (ref. 26), a derivative of pBR333. The resulting plasmid was integrated into pDPT51 by recombination *in vivo* to yield pDPT51:*LEU2*. All plasmids other than R751, pDPT51, pACYC184 and pRS2405 contain yeast 2μ-plasmid sequences which permit automomous replication in yeast.

* The plasmids contain genes which confer resistance to antibiotics in bacteria. Tp^r indicates that bacteria harboring the plasmid are resistant to trimethoprim; Am^r, resistance to ampicillin; Tc^r, resistance to tetracycline; and Cm^r, resistance to chloramphenicol. In addition, some plasmids contain the yeast *LEU2* or *URA3* genes. These plasmids can be selected in yeast because they complement nutritional auxotrophies conferred by mutations in the chromosomal *LEU2* or *URA3* genes.

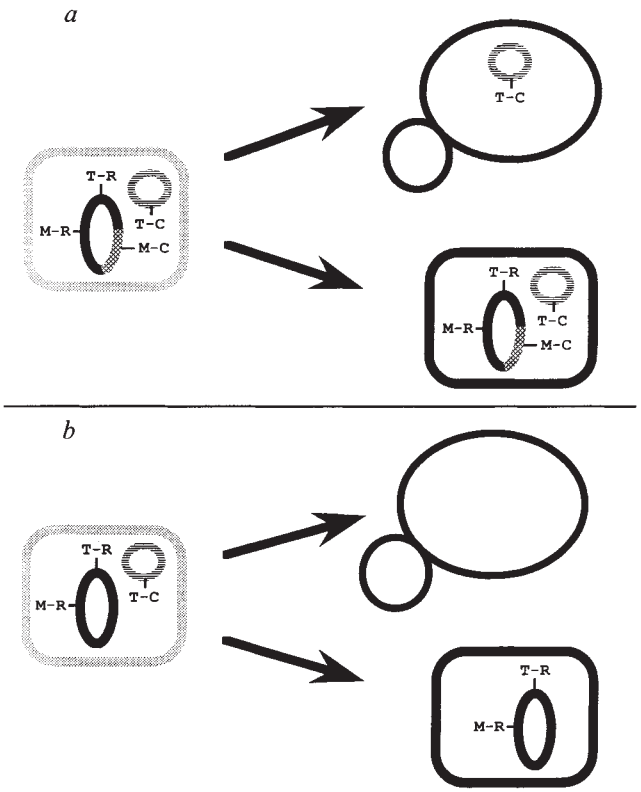

FIG. 1 Representation of the requirement for appropriate mobilization functions for plasmid transmission to bacteria and to yeast, as interpretated from results presented in Table 3, *a*1 and *a*2. The large rounded squares represent bacteria; donor bacteria are depicted with stippled borders and recipient bacteria with solid borders. The large budded circle represents yeast. Circles and ellipses within the cells represent plasmids. The ellipse with the solid border is plasmid R751. Plasmid pDPT51 is a derivative of R751 that carries the *mob*-C region (depicted by wide cross-hatching) from ColE1. The circle with the horizontally-striped border represents the bacteria–yeast shuttle vector YEp13. The *mob* and *oriT* functions present on the plasmids are represnted by abbreviations: M-R, *mob*-R; T-R, *oriT*-R; M-C, *mob*-C; T-C, *oriT*-C. *a*, The *mob*-C function of pDPT51 acts at the *oriT*-C of YEp13 and promotes its transmission to both bacteria and yeast. The *mob*-R function of pDPT51 acts at the *oriT*-R present on the same plasmid and promotes its transmission to bacteria. In principle, pDPT51 may also be transferred to yeast, but the plasmid lacks functions that permit replication and selection in yeast, so transfer cannot be detected. *b*, Plasmid R751 is transmitted to bacteria by means of its *mob*-R and *oriT*-R functions. However, YEp13 is not transmitted to either bacteria or yeast because no *mob*-C function is present in the donor bacterium. Again, R751 may also be transferred to yeast but this is undetectable.

TABLE 2 Physical requirements for plasmid transfer

Experiment (plasmids carried by donor)	Recipient*	Treatment	Transconjugant cells per ml	Donor: recipient ratio	Transconjugant cells per recipient
a					
YEp13	Y	none	0	30:1	$<1\times10^{-9}$
pDPT51, YEp13	Y	none	42	6:1	3×10^{-7}
b					
None	Y	YEp13 DNA	0	40:1	$<5\times10^{-9}$
pDPT51	Y	YEp13 DNA	0	30:1	$<1\times10^{-9}$
pDPT51, YEp13	Y	none	73	6:1	1×10^{-7}
No bacteria	Y	YEp13 DNA	0	—	$<2\times10^{-9}$
c					
pDPT51, YEp13	Y	$CHCl_3$	0	—	$<5\times10^{-10}$
pDPT51, YEp13	Y	$CHCl_3$ buffer	15	3:1	2×10^{-7}
pDPT51, YEp13	Y	none	11	1:2	3×10^{-8}
d					
pDPT51, YEp13	Y	pellet	3×10^{2}	1:300	3×10^{-6}
pDPT51, YEp13	B	pellet	2×10^{6}	1:30	2×10^{-1}
pDPT51, YEp13	Y	supernatant	0	—	$<2\times10^{-8}$
pDPT51, YEp13	B	supernatant	0	—	$<2\times10^{-7}$
e					
pDPT51, YEp13	Y	separated by filter	0	—	$<5\times10^{-9}$
pDPT51, YEp13	B	separated by filter	0	—	$<5\times10^{-10}$
pDPT51, YEp13	Y	combined on filter	1×10^{4}	1:30	5×10^{-5}
pDPT51, YEp13	B	combined on filter	2×10^{8}	1:2	3×10^{-1}

Donor and recipient cells were prepared for conjugation by a procedure adapted from Sistrom *et al.*[27]. Exponential cultures of donor bacteria growing in medium selective for plasmid maintenance were collected by centrifugation, washed with TNB (0.05 M Tris, pH 7.6, 0.05% NaCl), and resuspended in TNB (10^5–10^8 cells per ml). Similarly, exponential cultures of recipient cells growing in rich medium (LBH (1% tryptone, 0.5% NaCl, 0.5% yeast extract, 1 mM NaOH)[28] for bacteria, or YEPD (2% peptone, 1% yeast extract, 2% glucose)[29] for yeast) were collected and resuspended in TNB (10^8 cells per ml). Donor and recipient were mixed in the ratios indicated and plated as described below. Reported results are from representative experiments. Experiments *a*, *b* and *c* used bacterial strain RR1 (*proA2 leuB6 hsdS20 thi ara14 galK2 xyl5 mtl1 lacY1 supE44 rpsL20*; ref. 25) as the donor, and yeast strain DC5 (*MATa leu2-3 leu2-112 his3 gal2 can1*) as the recipient. Experiments *d* and *e* used bacterial strain SB21 (*hsdS leuB6 thr*, a derivative of C600; ref. 30) as the donor, SY1229 (*MATa leu2-3 leu2-112 his3 gal2 can1 ura3*) as the yeast recipient, and RR1 as the bacterial recipient. Two other yeast strains have also served as recipients, one of which is only distantly related to DC5 and SY1229. Experiment *a* established that the formation of Leu^+ yeast was dependent on a bacterial conjugation plasmid. The mixture of donor and recipient was plated directly on supplemented yeast minimal medium (SD (10.67% yeast nitrogen base, 2% glucose) + his (20 $\mu g\ ml^{-1}$ L-histidine); ref. 29). The number of transconjugants per ml was calculated on the basis of the volume of the mixture applied to the solid medium. No reversion to Leu^+ was detected. Incubation of the conjugation mixture on non-selective medium before plating gave comparable frequencies of Leu^+ transconjugants. Experiment *b* demonstrated that extracellular YEp13 DNA did not yield Leu^+ yeast. The donor–recipient mixture was preincubated on solid non-selective medium for 5–12 hours, resuspended in TNB, and plated on SD + his. The number of transconjugants per ml was calculated on the basis of their concentration in this final suspension of TNB. The concentration of YEp13 DNA in the mixture was 6 $\mu g\ ml^{-1}$. Experiment *c* showed that the formation of Leu^+ yeast was dependent on viable donor cells. The donor-recipient mixture was treated as in experiment *b*. Experiment *d* demonstrated that the formation of Leu^+ yeast was not caused by an extracellular diffusible factor. Pelleted donor cells or the supernatant of the donor culture was mixed with the yeast recipient and plated directly on SD + his + ura (20 $\mu g\ ml^{-1}$ uracil). All bacterial matings (also in Table 3) were first plated on LBH to allow expression of the antibiotic-resistant phenotype. After 5 h the cells were resuspended and plated on OMBG (minimal agar plates; ref. 31) plus proline (20 $\mu g\ ml^{-1}$), leucine (20 $\mu g\ ml^{-1}$) and trimethoprim (Tp) (200 $\mu g\ ml^{-1}$) and tetracyclin (Tc) (20 $\mu g\ ml^{-1}$). The frequency of transconjugant bacteria reflects the number of bacteria that received both plasmids. The same frequency was observed when transmission of only YEp13 was monitored. Experiment *e* confirmed that the formation of Leu^+ yeast required cell-to-cell contact. The mixture was preincubated on solid non-selective medium for 5–12 h and then plated on SD + his + ura. The bacterial matings were performed as in experiment *d*.

* Y indicates that the recipient organism was yeast; B indicates that the recipient organism was bacteria.

refer to the Leu^+ yeast obtained by co-culture with bacteria as 'transconjugants'.

DNA is transmitted through conjugation

We used two different approaches to decide whether plasmid DNA can be transmitted from bacteria to yeast by a mechanism equivalent to bacterial conjugation: the first involved physically disturbing the conjugal environment so as to disrupt bacterial conjugation, and the second was to disrupt genetically the yeast–bacteria system and to determine whether transmission needed the plasmid loci necessary for conjugation between bacteria.

Physical requirements for DNA transmission. DNA transfer between bacteria by conjugation is distinguished from transduction or transformation by a dependence on cell contact, donor-cell viability and residence of the plasmid in the donor cell at the time of cell-to-cell contact. It was conceivable that the transmission of YEp13 to yeast occurs by a process unrelated to bacterial conjugation. For example, bacteria with conjugation plasmids may lyse more frequently or increase by other means the concentration of extracellular DNA in their vicinity. In four separate experiments we have demonstrated that the transmission of YEp13 DNA from bacteria to yeast has the same physical requirements as bacterial conjugation: (1) Exogenously added plasmid is not transmitted to yeast cells. Yeast cells were subjected to the conjugation protocol in the presence of more YEp13 DNA than would be released if all bacterial cells in the bacteria–yeast conjugation system had lysed. The 'mock' conjugations were performed with YEp13 alone, with YEp13 and plasmid-free bacteria, or with YEp13 and *E. coli* containing only the pDPT51 plasmid. Leu^+ yeast cells were not observed in any of these experimental combinations (Table 2*b*). The same yeast culture was mixed with *E. coli* cells containing both pDPT51 and YEp13 as a positive control, and Leu^+ transconjugant yeast were evident. We also demonstrated that extracellular DNA could not be responsible for the occurrence of Leu^+ yeast by adding DNaseI to the bacteria–yeast mixture, and we found no interference with DNA transmission (data not shown; ref. 13). (2) Viable donor cells are required. A culture of *E. coli* containing pDPT51 and YEp13 was split into three. The first sample was treated with chloroform to kill the cells, and the chloroform then removed[14]. The second sample was treated with buffer prewashed with chloroform in the same way as the first to test the efficiency of chloroform removal. The third sample was untreated. Leu^+ yeast cells were not recovered when the chloroform-treated cells were used as donors, but were with the buffer-treated or untreated cells (Table 2*c*). (3) DNA transmission does not involve an extracellular diffusible factor. A

TABLE 3 Genetic requirements for plasmid transfer

Experiment (plasmids carried by donor)	Recipient*	Selection	Transconjugant cells per ml	Donor: recipient ratio	Transconjugant cells per donor
*a*1					
pDPT51 (*mob*-C, *mob*-R, *oriT*-R, Tp^r)	Y	Leu$^+$	5×10^3	1:1,000	7×10^{-2}
YEp13 (*oriT*-C, Tc^r, *LEU2*)	B	Tp^r	4×10^6	1:6,000	2
	B	Tp^r+Tc^r	5×10^6	1:6,000	3
	B	Tc^r	4×10^6	1:6,000	2
*a*2					
R751 (*mob*-R, *oriT*-R, Tp^r)	Y	Leu$^+$	0	1:100	$<2\times10^{-5}$
YEp13 (*oriT*-C, Tc^r, *LEU2*)	B	Tp^r	4×10^6	1:4,000	2
	B	Tp^r+Tc^r	0	1:4,000	$<2\times10^{-6}$
	B	Tc^r	0	1:4,000	$<2\times10^{-6}$
*b*1					
pDPT51 (*mob*-C, *mob*-R, *oriT*-R, Tp^r)	Y	Ura$^+$	1×10^3	5:1	3×10^{-5}
YEp24 (*oriT*-C, Tc^R, *URA3*)	Y	Leu$^+$	0	5:1	$<3\times10^{-7}$
pAL2 (Cm^r, *LEU2*)	B	Tp^r	2×10^7	1:6	6
	B	Tc^r	2×10^6	1:6	4×10^{-1}
	B	Cm^r	4×10^4	1:6	8×10^{-3}
*b*2					
pDPT51 (*mob*-C, *mob*-R, *oriT*-R, Tp^r)	Y	Leu$^+$	1×10^2	30:1	3×10^{-7}
pAL2 (Cm^r, *LEU2*)	B	Tp^r	3×10^5	3:1	3×10^{-1}
	B	Cm^r	8×10^7	3:1	3×10^{-3}
*b*3					
pDPT51 (*mob*-C, *mob*-R, *oriT*-R, Tp^r)	B	Tp^r	2×10^6	1:1	1×10^{-1}
pACYC184 (Cm^r)	B	Cm^r	0	1:1	$<5\times10^{-8}$
*c*1					
pFLEU2 (*mob*-F, *oriT*-F, *LEU2*)	Y	Leu$^+$	1×10^2	5:1	5×10^{-8}
*c*2					
pDPT51::*LEU2* (*mob*-R, *oriT*-R, *mob*-C, *LEU2*)	Y	Leu$^+$	1×10^2	3:1	1×10^{-7}
*c*3					
R751 (*mob*-R, *oriT*-R)	Y	Leu$^+$	0	6:1	$<3\times10^{-10}$
YEp13 (*oriT*-C, Tc^r, *LEU2*)	B	Tp^r	1×10^8	2:1	5×10^{-1}
	B	Tc^r	0	2:1	$<3\times10^{-9}$

Conjugation assays were performed as described for Table 2. During the course of these experiments it was discovered that a donor to recipient ratio of ~1:1,000 (10^4–10^5 donor cells per ml and 10^7–10^8 recipient cells per ml) resulted in a higher frequency of transmission than ratios closer to 1:1 (10^8 cells per ml of both types). Experiments reported are representative, not an average of multiple experimental results. The *a* set of experiments demonstrated that plasmid transmission was dependent on *mob* activity. The donor bacterial strain was SB21, the recipient yeast strain was SY1229, and the recipient bacterial strain was RR1. Leu$^+$ transconjugant yeast cells were selected by direct plating of the conjugation mixture on SD+his+ura (to select YEp13). Transconjugant bacteria were selected on OMBG+pro+leu+streptomycin (Sm; 100 μg ml^{-1}) medium which also contained either Tp alone (to select pDPT51 or R751) or both Tp and Tc (to select pDPT51 and YEp13). Transconjugant bacteria were also selected on LBH+Sm+Tc (to select YEp13). The *b* set of experiments established the specificity of the *mob*-*oriT* interaction. The donor bacterial strain was DH1 (*recA1 endA1 gyrA96 thi-1 hsdR17 supE44*; ref. 32), the recipient bacterial strain was HB101 (*recA* derivative of RR1; ref. 25), and the recipient yeast strain was SY1229. Transconjugant yeast cells were selected by direct plating on SD+his+ura (to select pAL2) or on SD+his+leu (to select YEp24). Transconjugant bacteria were selected by plating on OMBG+pro+leu+Sm+Tp (to select pDPT51); on LBH+Sm+Tc (to select YEp24); or on LBH+Sm+chloramphenicol (Cm; 12 μg ml^{-1}) (to select pAL2 or pACYC184). Although there was no pAL2 transmission to yeast in our experiment *b*1, in repeat experiments it occurred at a frequency of 3×10^{-7}. The *c* set of experiments demonstrated that derivatives of the F plasmid were capable of promoting DNA transfer to yeast. The donor bacterial strain was DH1 (*c*1 and *c*2) or SB21 (*c*3), the recipient bacterial strain was HB101, and the recipient yeast strain was SY1229. Transconjugant yeast cells were selected by preincubation on solid non-selective medium for 5–12 h before plating on SD+his+ura (to select pFLEU2, pDPT51::LEU2, or YEp13). Transconjugant bacteria were selected by plating on OMBG+pro+leu+Sm+Tp (to select R751), or on LBH+Sm+ampicillin (Am; 100 μg ml^{-1}) (to select YEp13).

* Y indicates that the recipient organism was yeast; B indicates that the recipient organism was bacteria.

culture of *E. coli* containing pDPT51 and YEp13 was divided into supernatant and pellet fractions by filtration through a 0.45-μm pore Millipore filter and each fraction mixed with a yeast culture in a conjugation assay. Leu$^+$ transconjugant yeast cells were recovered from the mixture of pellet and yeast, but no Leu$^+$ transconjugants were recovered when the yeast cells were mixed with the supernatant fraction (Table 2*d*). (4) Cell contact is required for DNA transmission. Yeast and donor bacteria were applied to the surface of a 0.45-μm pore Millipore filter in two different orientations. In one orientation the yeast and bacteria were on the same side of the filter and in the other, the filter separated them. Leu$^+$ transconjugant yeast cells were recovered only when the yeast and bacteria were on the same side of the filter (Table 2*e*).

Transconjugant yeast cells were recovered only when the conjugation plasmid and YEp13 were present in a viable donor and when cell contact was allowed, thus demonstrating that the requirements were the same as for bacterial conjugation.

Genetic requirements for DNA transmission. We investigated whether DNA transmission required the conjugation functions *mob* and *oriT*. If the transmission of DNA from bacteria to yeast used the *mob* and *oriT* functions required for bacterial conjugation, then the transmission process should discriminate between plasmids using the same rules for *mob*/*oriT* interaction that characterize bacterial conjugation.

To test whether the ColE1 *mob* functions were necessary for transmission of YEp13 from bacteria to yeast, we compared the ability of *E. coli* cells containing either pDPT51 or R751 to transmit YEp13 to yeast. As noted above, pDPT51 contains *mob*-C, whereas its parent, R751, does not (Table 1); also, pDPT51 promotes transfer of YEp13 to yeast whereas R751 does not (Table 3, *a*1 and *a*2; Fig. 1). Matings of these donor bacteria with other bacteria verifies that the R751 and pDPT51 conjugation systems are operative and have the properties expected. As shown in Table 3, *a*1 and *a*2, *E. coli* containing pDPT51 and YEp13 can transmit both plasmids, whereas *E. coli* contain-

ing R751 and YEp13 can transmit only R751 to the recipient bacteria. We conclude that transmission of plasmid DNA from bacteria to yeast requires *mob* functions that are compatible with the *oriT* on the yeast plasmid.

The importance of *oriT* in DNA transmission from bacteria to yeast was tested by comparing transmission of *oriT*-C^+ and *oriT*-C^- plasmids co-resident in a bacterial strain. We constructed a bacterial strain carrying pDPT51 (to provide *mob*-C functions) as well as *oriT*-C^+ or *oriT*-C^- yeast plasmids (YEp24 and pAL2, respectively). As shown in Table 3, *b*1, transmission of the *oriT*-C^+ plasmid to yeast recipients is increased over that of the *oriT*-C^- plasmid by a factor of ~100. Bacterial matings gave the same relative ratio of transmission of the two plasmids. We did not expect pAL2 to exhibit any transmission to either yeast or bacteria because its parent plasmid, pACYC184, is strongly *oriT*-C^- and cannot be transmitted in bacterial matings (Table 3, *b*3; ref. 15). Evidently the *LEU2* or 2μ sequence in pAL2 has weak *oriT*-C activity or can undergo site-specific recombination with a plasmid present in the bacterial donor. The salient observation is, however, that the *oriT*-C^+ plasmid is transmitted at a much higher frequency than the *oriT*-C^- plasmid.

We then established that differences in plasmid copy number or competition for transfer machinery could not be responsible for the different transmission frequencies of YEp24 and pAL2. DNA was extracted from the donor cells and used to transform *E. coli*[16]. Chloramphenicol- (pAL2) and tetracycline-(YEp24) resistant transformants were obtained at nearly equal frequencies (data not shown). Also, a donor containing only pDPT51 and pAL2 transmitted pAL2 to yeast and bacteria at essentially the same frequency as the bacterial strain containing pDPT51, pAL2 and YEp24 (compare Table 3, *b*1 and *b*2). Thus, efficient transmission of a plasmid from bacteria to yeast seems to require that the plasmid have an *oriT* that can be acted on by *mob* gene products present in the donor cell.

F factor mediates conjugation with yeast

The experiments described so far rely on cell contact and transfer functions encoded by the broad-host-range plasmid R751 and its derivative pDPT51, both of which promote conjugation between *E. coli* and many other bacterial species. The F plasmid, however, confers a limited-host-range phenotype. We tested whether the apparent limited-host-range of such a plasmid could be extended to include yeast by the incorporation of a sequence which would allow it to replicate autonomously in yeast. This approach has already shown that derivatives of F can be transmitted to *Pseudomonas aeruginosa* when they contain a sequence that confers autonomous replication in that species[8].

Plasmid pRS2405 (Table 1), a derivative of F (refs 9 and 10) that carries cell contact and transfer functions from F (*tra*-F) as well as *mob*-F and *oriT*-F, was engineered to carry the yeast *LEU2* gene and the 2μ replication sequence (pFLEU2; Table 1). To compare directly the transmission of the F plasmid to the R751-based plasmids, we constructed a derivative of pDPT51, pDPT51:LEU2, that could be monitored in yeast by virtue of *LEU2* and 2μ sequences. *E. coli* cells containing one or the other plasmid were able to conjugate with yeast to yield Leu^+ transconjugants (Table 3, *c*1 and *c*2), although transmission was less than that measured for YEp13 co-resident with pDPT51 (Table 3, *a*1). Both pFLEU2 and pDPT51:LEU2 (>50 kilobase pairs (kb)) are larger than YEp13 (11 kb) and so could either be transmitted less efficiently or have more difficulty in becoming established in yeast. Whatever the explanation, transmission of pFLEU2 and pDPT51:LEU2 reflects conjugal transmission to yeast, because we detected no transconjugant yeast cells in a control cross involving donor cells which could not transmit YEp13 (Table 3, *c*3).

Conclusions

We have found that bacterial plasmids that mediate conjugation between bacterial cells are also able to mediate plasmid transmission to the yeast *S. cerevisiae* by a process that seems, by our physical and genetic criteria, to be conjugation. Of the genetic functions required for conjugation between bacteria, we have shown that *mob* and *oriT* are necessary for bacteria-yeast conjugation. It remains to be determined whether *tra* functions required for cell-surface interactions in bacterial conjugation also participate in bacteria-yeast conjugation. Nonetheless, it is clear that two distinct cell-contact and transfer functions, one encoded by R751 and the other by F, and two *mob*/*oriT* systems, *mob*-C/*oriT*-C and *mob*-F/*oriT*-F, are capable of promoting DNA transmission from bacteria to yeast.

The conjugation functions encoded by the conjugative plasmids can apparently form contacts competent for DNA transfer with organisms separated by large evolutionary distances. Our results suggest that conjugation could occur between bacteria and animal cells and between bacteria and plant cells, even when the bacteria do not carry the specialised tumour-inducing plasmid. The conjugation system we have studied here provides a mechanism for the transfer of DNA between distantly related species. Such lateral transmission could be a significant factor in evolution and may help to explain discrepancies in phylogenic trees[17,18].

Finally, our system provides a rapid method, which in some cases could be superior to DNA transformation, for introducing plasmids into yeast and possibly into other organisms. Moreover, the system may provide a tractable way to investigate the molecular mechanism of transfer of DNA from *A. tumefaciens* to plant cells should it be possible to substitute yeast for the plant component. □

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