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Positive Selection for *Dictyostelium* Mutants Lacking Uridine Monophosphate Synthase Activity Based on Resistance to 5-Fluoro-orotic Acid

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ABSTRACT Whereas transformation of *Dictyostelium discoideum* now can be done routinely and reliably, there is increasing demand for a system that allows selection of cells that have gone through repeated transformation cycles. Such a system is presented here. Selection is based on resistance to 5-fluoro-orotic-acid (5-FOA). *D. discoideum* is highly sensitive to this drug, and cells can survive only in the presence of 5-FOA if they carry a defective UMP-synthase gene. After transformation with a plasmid carrying a cloned version of the UMP-synthase gene, 5-FOA resistant cells are obtained at high frequency. Because 5-FOA-resistant cells depend on exogenously added uracil, these cells can be taken through a second round of transformation. A plasmid-borne UMP-synthase gene renders 5-FOA-resistant cells phenotypically ura^+. Finally, cells can be further transformed using the standard G418 selection system.

Key words: Transformation, *Dictyostelium discoideum*, UMP-synthase/ura^- complementation

INTRODUCTION

Although DNA can be efficiently introduced into eukaryotic cells, there are still only a few selection systems available that allow identification of transformed cells that have taken up recombinant DNA molecules. Such selection systems are based, e.g., on thymidine kinase (TK) [Wilkie *et al.*, 1979], hypoxanthine guanine phosphoribosyl transferase (HGPRT) [Mulligan and Berg, 1981], adenoribosyl transferase (APRT) [Wigler *et al.*, 1979], or dihydrofolate reductase (DHFR) [Alt *et al.*, 1978]. Additionally, some antibiotics can be used for selection; however, their use is restricted to a very small number of highly toxic compounds such as the aminoglycoside G418 and chloramphenicol. In these cases, prokaryotic gene products, i.e. aminoglycoside 3′-phosphotransferase [Davies and Smith, 1978] and chloramphenicol acetyl transferase [Shaw, 1967], are responsible for the detoxification of the drugs.

Currently most transformation vectors which are used for *D. discoideum* transformation rely on the neomycin or kanamycin resistance genes derived either from Tn5 or Tn903 [Nellen *et al.*, 1984; Knecht *et al.*, 1986]. As transformation using G418 selection is now routine, there is increasingly need for additional selection systems. Ideally these should work with any axenic strain, rather than specialized mutants, and they should not interfere with G418 selection. Such a system will be described.

MATERIALS AND METHODS

Transformation and Selection Procedures

D. discoideum Ax-2 cells were routinely grown in HL5 medium [Watts and Ashworth, 1970] at 22°C. Cells were transformed by standard procedures [Nellen et al., 1984; Early and Williams, 1987] with the following modifications. For 5-FOA selection, cells were always grown in medium containing uracil (20 μg/ml). 5-FOA (50–100 μg/ml, obtained from Sigma #F5013) was added when changing medium the day after transformation. Medium was replaced by fresh selection medium every second day. Primary colonies were picked from the Petri dish and recloned on SM-agar plates (containing 20 μg/ml uracil) with *Klebsiella aerogenes* [Welker and Williams, 1982]. After 3 to 5 days of growth at 22°C, single colonies were transferred to Costar plates and reselected in the presence of 50 to 100

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μg/ml 5-FOA. Resistant cells do have a *ura*$^-$ phenotype and grow significantly slower than wild-type cells.

For *ura*$^-$ complementation, deletion mutants are transformed with pDU3B1 or pUCPYR5-6. Transformants can be selected in HL5 medium. However, due to uracil's presence in this medium, a significant amount of untransformed cells interferes with an easy detection of transformants. On the day after the transformation, the medium should be changed and not replaced with fresh medium for at least 5 days. If clones become visible (usually 7–10 days after transformation), they should be recloned on SM-agar plates with *K. aerogenes* and then reselected on Costar plates in HL5 medium. At this stage, *ura*$^+$ cells can easily be distinguished from untransformed *ura*$^-$ cells by their significantly increased growth rate. Instead of HL5 medium, defined media [Franke and Kessin, 1977; Rahmsdorf, 1977] can be used for selection of *ura*$^+$ transformants. In such media, selection of positively transformed cells is unambiguous.

Preparation of DNA and RNA

Genomic DNA was prepared from isolated nuclei [Dingermann *et al.*, 1987]. Nuclei were lysed at 65°C in 8 ml buffer containing 200 mM EDTA, pH 8.4, and 2% sarcosyl. CsCl (8 g) plus 200 μl ethidium bromide (10 mg/ml) were added; after centrifugation (42,000 rpm, 20°C, 48 h), DNA was collected and ethanol precipitated and dissolved in Low TE buffer (10 mM Tris/HCl pH 8, 0.1 mM EDTA).

Total cellular RNA was prepared from 5×10^7 to 1×10^8 washed cells. Cells were lysed in 3.5 ml 4M guanidinium isothiocyanate and RNA pelleted through a cushion of 1.5 ml of 4.7M CsCl (35,000 rpm, 18°C, 16 h) dissolved in 360 μl TES buffer (10 mM Tris/HCl, pH 7.4, 5 mM EDTA, 1% SDS). After precipitation with 2.5 volumes of ethanol at −80°C, the purified RNA was stored in sterile water at −80°C.

RESULTS AND DISCUSSION

Theoretical Basis for 5-FOA Selection

Initial selection is based on resistance to 5-fluoro-orotic acid (5-FOA). This compound is toxic for nearly every organism. In normal cells, this drug is metabolized by UMP synthase, yielding 5-fluoro-uridine monophosphate (UMP), which covalently and irreversibly binds thymidylate-synthase (Fig. 1). This reaction is called suicide inhibition, because the enzyme is inactivated by binding to its substrate, and inactivation of thymidylate-synthase eventually causes cell death. However, cells lacking UMP-synthase activity are resistant to 5-FOA because the compound cannot be converted to 5F-dUMP, the substrate for thymidylate-synthase. Due to the UMP-synthase defect, cells need to be complemented with exogenously added uracil, which is converted by salvage pathways to UMP and further by the action of thymidylate synthase to thymidine monophosphate (TMP).

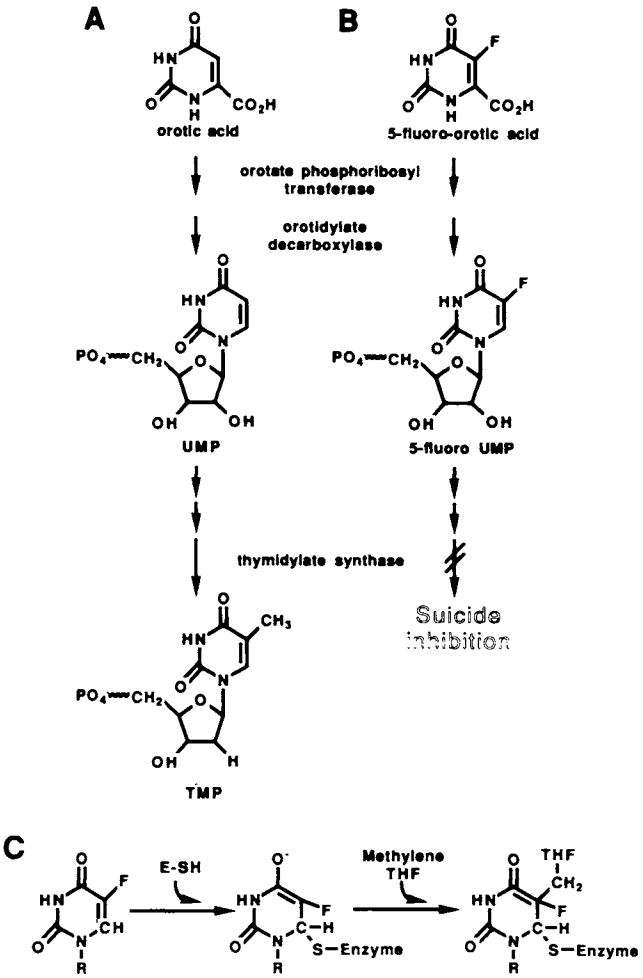

Fig. 1. Suicide inhibition. **A:** Orotic acid is one intermediate required for TMP biosynthesis. It is converted by two enzymatic activities (orotate phosphoribosyl transferase and orotidylate decarboxylase) yielding UMP. In *D. discoideum* both activities are represented by a single bifunctional enzyme referred to as *UMP synthase*. UMP is further converted via dUMP to thymidine monophosphate (TMP) by the enzyme thymidylate synthase. **B:** In the presence of 5-FOA, UMP synthase converts this compound to 5-fluoro-UMP, which after reduction to 5F-dUMP also acts as a substrate for thymidylate synthase. **C:** Thymidylate synthase is irreversibly inhibited by 5F-dUMP. Because F^+ cannot be abstracted from F-dUMP by the enzyme, catalysis is blocked at the stage of the covalent complex formed by F-dUMP, methylenetetrahydrofolate, and the sulfhydryl group of the enzyme. Cells are rapidly depleted of active enzyme and die. This effect of 5-FOA, known as *suicide inhibition*, can be prevented if cells are unable to convert 5-FOA to 5F-UMP, i.e., if they do not contain a functional UMP synthase.

Conversion of orotic acid to UMP is generally achieved by the action of two enzymatic activities: orotate phosphoribosyl transferase (EC 2.4.2.10) and orotidine-5′-phosphate carboxy-lyase (= orotidylate decarboxylase) (EC 4.1.1.23). Whereas in prokaryotes—but also in some lower eukaryotes like *S. cerevisiae*—each

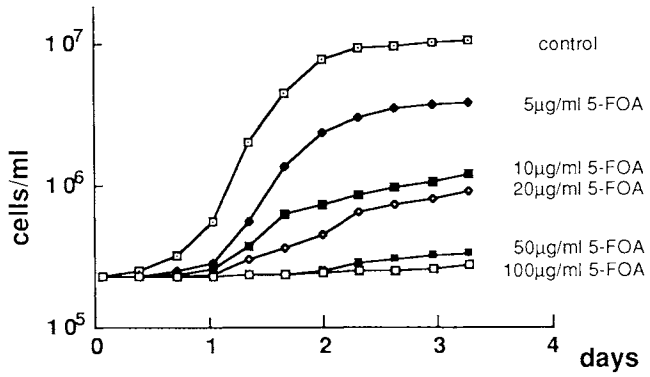

Fig. 2. Growth inhibition of *D. discoideum* cells by 5-FOA. Ax-2 cells were grown in shaking cultures at 22°C. Indicated amounts of 5-FOA were added to the cultures, and cells were counted at indicated intervals. Cells grown on Petri dishes are slightly less sensitive to 5-FOA than cells grown in shaking cultures (data not shown).

activity represents a separate enzyme, *D. discoideum* as well as *Drosophila melanogaster* and mammals encode both enzymatic activities on a single gene. This gene was isolated from *D. discoideum* by screening a genomic library in a *ura*3 defective background of *Saccharomyces cerevisiae* [Boy-Marcotte and Jacquet, 1982]. The gene is 1.8 kb long and is also able to complement a *ura*5 mutation in yeast [Boy-Marcotte *et al.*, 1984]. In addition to this genetic evidence, significant homologies on the amino acid level confirmed the suggestion that in *D. discoideum* UMP-synthase is encoded by a single gene [Jacquet *et al.*, 1988].

Selection of 5-FOA Resistant Ax-2 Cells After Transformation With a Plasmid Carrying the DdPYR5-6 gene. *D. discoideum* cells are highly sensitive to 5-FOA. In the presence of 50 to 100 μg 5-FOA/ml HL5-medium, cell growth is completely inhibited in shaking cultures (Fig. 2). Obviously, *D. discoideum* is much more sensitive to the drug than, e.g., *S. cerevisiae,* where concentrations of 2 mg/ml are needed for efficient growth inhibition [Boeke *et al.*, 1984]. It should be pointed out, however, that cells grown in Petri dishes seem to be slightly less sensitive. In this case, the addition of 100 μg 5-FOA per ml HL5-medium is required for complete growth inhibition.

To isolate mutants carrying a defective UMP-synthase gene, cells were transformed with pDU3B1 [Boy-Marcotte *et al.*, 1984], a plasmid containing the entire UMP-synthase gene (Fig. 3). Transformation was done following the standard protocols [Nellen and Firtel, 1985; Nellen *et al.*, 1984, 1986; Early and Williams, 1987], with the exception that all media contained 20 μg/ml uracil and that the day following the transformation 100 μg/ml 5-FOA was added. Medium was changed every second day, and resistant cells were visible at around day 5 to day 7 after transformation. Selection was continued for another 7 days, and apparently resistant cells were cloned on bacterial plates.

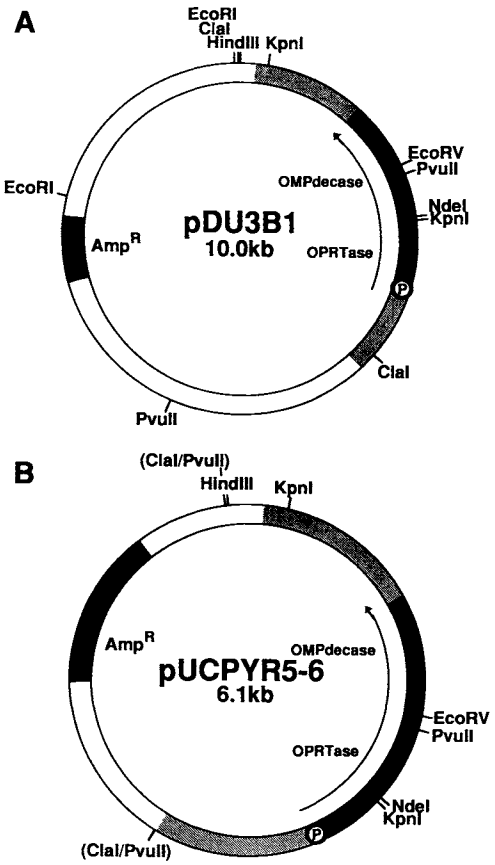

Fig. 3. Schematic drawings of plasmids pDU3B1 and pUCPYR5-6. The construction of pDU3B1 is described in detail in Boy-Marcotte et al. (1984). The plasmid contains a *Cla*I fragment of about 3.7kb, which encodes the entire UMP-synthase gene. Note that the *Cla*I site, which is flanked by *Eco*RI and *Hin*dIII restriction sites, originates from vector sequences and does not represent genomic DNA. The location of the promoter and the transcription direction is indicated. Plasmid pUCPYR5-6 was constructed by a blunt-end ligation of the *Cla*I fragment from pDU3B1 into a *Pvu*II digested pUC vector. pUCPYR5-6 contains a unique *Pvu*II site within the coding region. This can conveniently be used to insert a DNA fragment, which may be directed into the DdPYR5-6 site of the *D. discoideum* genome.

Careful recloning is very important. In the presence of 100 μg/ml 5-FOA, many untransformed cells are not killed; instead, they are only growth inhibited. This is the reason for high background levels during 5-FOA selection. These untransformed cells start growing again as the drug is removed and quickly overgrow mutant cells. Therefore, clones picked from a bacterial plate have to be reselected in 5-FOA selection medium. This time false-positive transformants are readily detectable and can be discarded.

Transformation efficiencies were unexpectedly high. Usually, 50 or more independent transformants are obtained per 10^7 input cells, which in our hands is not significantly less than obtained after G418 selection. This was surprising, as 5-FOA selection depends on gene targeting and subsequent gene inactivation while

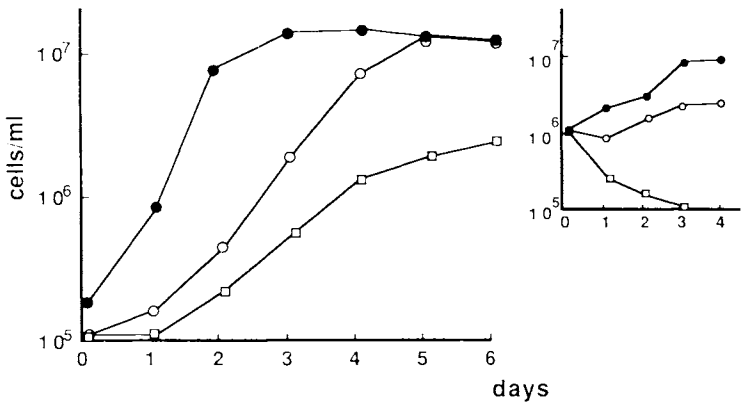

Fig. 4. Growth characteristics of Ax-2 cells and of the N1-2 deletion mutant in HL5-medium and in defined medium (insert). Ax-2 cells (closed circles) grow identically in HL5-Medium or in defined medium whether or not uracil at a concentration of 20 μg/ml is present. N1-2 cells, however, depend very much on exogenously added uracil. In HL5-medium plus uracil (20 μg/ml), N1-2 cells grow significantly more slowly than Ax-2 cells, but they eventually reach similar cell densities (open circles). If uracil is omitted, N1-2 cells grow even more slowly and stop growing at about 2×10^6 cells/ml (open squares). In defined medium, N1-2 cells grow slowly in the presence of uracil (insert, open circles) but start immediately dying if cultured in uracil-free defined medium (insert, open squares).

the *neo*^{r} gene can integrate anywhere in the genome. On the other hand, G418 resistance usually requires amplification of the *neo*^{r} gene, which seems to be a rare event as well (unpublished observation).

Although vectors used in this study are not real "disruption plasmids," 5-FOA-resistant cells seem to be the result of gene disruption through specific targeting by the transformation plasmid. From mock transformations and from transformations with plasmids carrying no DNA fragments homologous to the UMP-synthase locus, only rarely are 5-FOA-resistant cells obtained. We speculate that plasmids integrate into the UMP-synthase gene first. In the presence of 5-FOA, clones are then selected that have deleted this DNA together with parts of the resident gene. Experiments with "disruption plasmids" are currently in progress.

Phenotypic analysis of mutants confirmed the suggestion that 5-FOA-resistant cells also exhibit an *ura*$^-$ phenotype. Furthermore, growth rate is greatly reduced. In the presence of 20 μg/ml uracil, 5-FOA-resistant cells grow with a generation time of about 16 hours, but finally reach cell densities of untransformed Ax-2 cells (Fig. 4). Without uracil added to the HL5 medium, 5-FOA-resistant cells stop growing in shaking cultures at a cell density of about 2×10^6 cells/ml (Fig. 4). The *ura*$^-$ phenotype is even more obvious if cells are grown in defined medium [Rahmsdorf, 1977; Franke and Kessin, 1977]. If this medium is not supplemented with uracil, cells start dying immediately (Fig. 4, insert).

Selection of *ura*$^+$ cells Following Transformation of 5-FOA-resistant cells With a Plasmid Carrying the DdPYR5-6 gene. The obvious *ura*$^-$ phenotype of 5-FOA-resistant cells suggested another selection scheme after a second round of transformation. Cells were transformed with pDU3B1, and transformants were selected either in defined medium or in HL5 medium without any uracil added. Although selection in defined medium was unambiguous and effective, selection in HL5 medium was slightly complicated by the fact that untransformed *ura*$^-$ cells were able to grow in this medium to some degree. In this case, medium should be changed only every 5th day, and clones that become detectable after about 10 days need to be carefully rescreened by recloning on *K. aerogenes* followed by growth in HL5 medium without uracil. Resulting transformants are clearly distinguishable from *ura*$^-$ cells not only because of their *ura*$^+$ phenotype, but also because of growth characteristics comparable to those of wild-type cells.

Genotypic Analysis of Ax-2 cells, 5-FOA Resistant Transformants and ura$^+$ Supertransformants. To gain insight into the genetic changes that led to the two new phenotypes, genomic DNA as well as RNA were isolated from Ax-2 cells, from cells selected based on 5-FOA resistance, and from cells obtained in a secoud round of transformation based on *ura*$^+$ selection. A comprehensive analysis is shown in Figure 5. Genomic DNAs were digested with *Kpn*I or with *Kpn*I/*Pvu*II and, after size fractionation and transfer to nitrocellulose, filters were hybridized with nick-translated pDU3B1 plasmid (Fig. 5A). It is obvious that signal intensities of most DNA fragments are comparable, indicating that significant gene amplification did not occur. Furthermore, it is obvious that the signal pattern in all three analyzed DNAs is different, indicating that some genomic rearrangement has taken place.

From extensive analyses we can conclude that in the analyzed 5-FOA-resistant mutant N1-2 a 0.7 kb spanning deletion occurred, most likely resulting from homologous recombination with pDU3B1 and subsequent

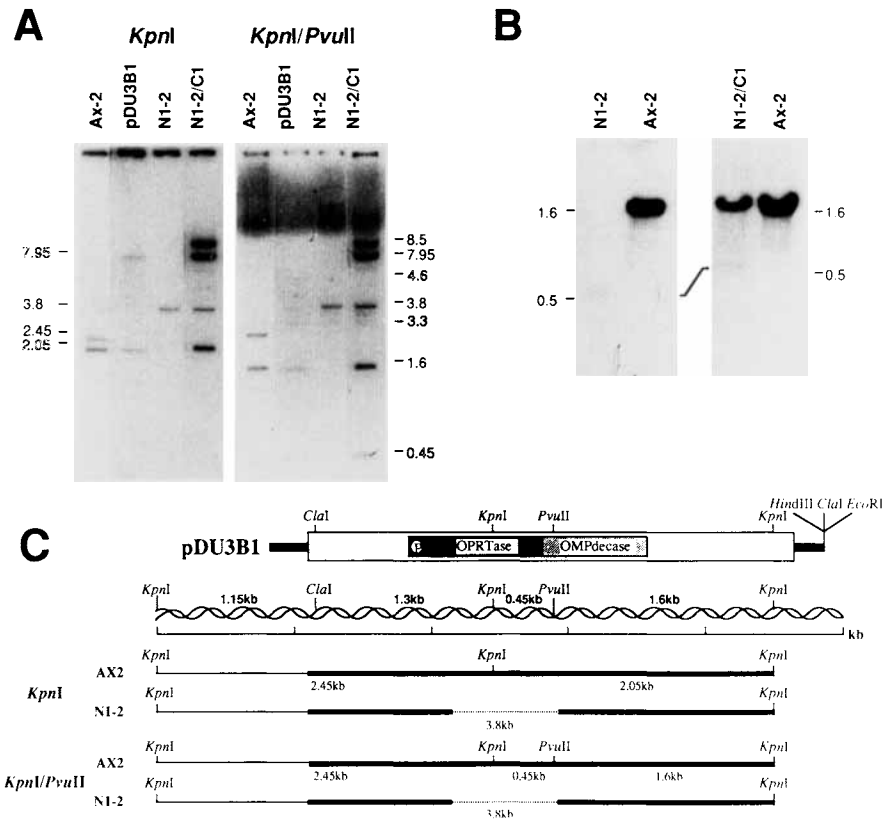

Fig. 5. A: Southern blot analysis of *Kpn*I- and *Kpn*I/*Pvu*II-digested nuclear DNA from Ax-2 cells, 5-FOA^r N1-2 cells, and *ura*$^+$ selected N1-2/C1 cells. As control, pDU3B1 plasmid DNA was analyzed as well. Blots were hybridized with nick-translated pDU3B1 plasmid DNA. According to these and additional analyses, N1-2 cells are characterized by a deletion of about 0.7 kb within the DdPYR5-6 locus (shown in **C**). There is no indication of any integrated plasmid DNA. N1-2/C1 cells, which were selected based on *ura*$^-$ complementation, most likely contain, in addition to the genomic deletion, one pDU3B1 plasmid integrated into the genome. Sizes of restriction fragments are given in kilobase pairs. Restriction fragments resulting from pDU3B1 are indicated by outlined lettering. **B:** Northern blot analysis of RNA isolated from Ax-2 cells, N1-2 cells, and N1-2/C1 cells. Ax-2 cells express a DdPYR5-6 mRNA of about 1.6 kb. The N1-2 deletion clone contains marginal amounts of a 0.5 kb mRNA, which hybridizes with the probe; N1-2/Cl contain both the intact 1.6 kb mRNA and the truncated 0.5 kb message.

imperfect deletion of the integrated and probably amplified plasmid DNA. This deletion effects the N-terminal coding part of the DdPYR5-6 gene, possibly including the promoter region (Fig. 5C), as both the internal *Kpn*I site and the internal *Pvu*II site are deleted in the mutant. As a consequence, 3.8 kb genomic DNA fragments can be detected in *Kpn*I as well as in *Kpn*I/*Pvu*II digested N1-2 DNA. Corresponding restrictions of Ax-2 control DNA result in fragments of 2.45 kb/2.05 kb and 2.45 kb/1.6 kb/0.45 kb, respectively. Unaffected, however, is the C-terminal part of the gene as well as sequences upstream of the coding region, as the right-most *Kpn*I site and the left-most *Cla*I site located on pDU3B1 are still present in the genome (data not shown). Clone N1-2 does not contain any plasmid DNA (Fig. 5A).

From the fragment pattern of clone N1-2/C1, it is obvious that both the wild-type and the deletion pattern appear to be superimposed. This result indicates that the *ura*$^+$ phenotype does not result from a gene conversion at the deleted DdPYR5-6 locus. Rather, a new DdPYR5-6 gene copy has been reintroduced into the mutant genome. This copy is present on the pDU3B1 plasmid, which has been entirely integrated into the mutant genome. Although slightly stronger signal intensities might suggest amplification of the integrated plasmid DNA, the following facts argue in favor of a single-copy integration. As a result of amplification of pDU3B1 DNA, the genomic restriction pattern should be identical to the pattern of correspondingly digested plasmid DNA; this is not observed. In addition to the predicted 7.95 kb and 2.05 kb *Kpn*I fragments, N1-2/C1 DNA contains another fragment of about 8.5 kb, which is of an intensity similar to all the other fragments, with the exception of the 3.8 kb fragment. This 3.8 kb fragment, however, carries the genomic deletion, which causes less efficient hybridization with the radioactive probe. Similarly the patterns

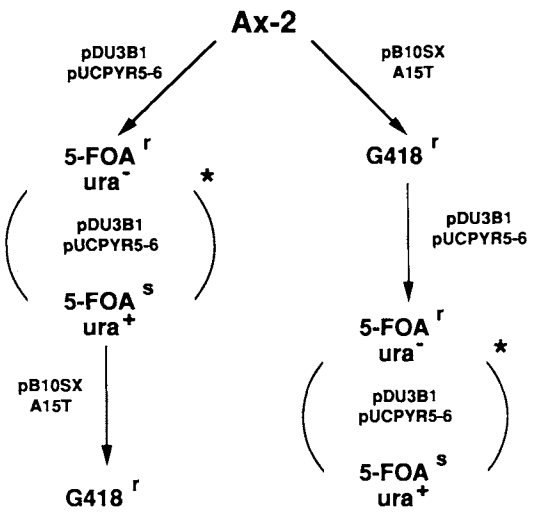

Fig. 6. A complex scheme which allows selection for *D. discoideum* transformants after repeated rounds of transformation. Starting from Ax-2 cells, initial transformants can be selected based on either G418 resistance or on 5-FOA resistance. 5-FOA-resistant cells, which are additionally characterized by a *ura*$^-$ phenotype, can be supertransformed with pDU3B1 or pUCPYR5-6, and transformants can be selected based on their *ura*$^+$ phenotype. Mostly one and only one copy of the plasmid is integrated into the genome, which seems sufficient to complement the *ura*$^-$ phenotype. Such strains (indicated by *) can be taken through another round of DdPYR5-6 gene disruption and *ura*$^-$ complementation.

of pDU3B1 plasmid DNA and N1-2/C1 genomic DNA are different after *Kpn*I/*Pvu*II double digest.

Similar results have been obtained with independently isolated transformants. All 5-FOA-resistant clones analyzed to date were characterized by a disrupted DdPYR5-6 gene and never contained any plasmid DNA. All analyzed clones obtained by *ura*$^+$ selection showed a mosaic genotype, and the majority contained one and only one copy of pDU3B1 integrated in the genome.

The DNA data are confirmed by RNA analyses. Although wild-type cells express a 1.6 kb message derived from the DdPYR5-6 gene, mRNA isolated from the 5-FOA resistant mutant N1-2 contains marginal amounts of a 0.5 kb mRNA (Fig. 5B). This supports the notion that the delection in the N1-2 genome affects the promoter region as well. N1-2/C1 cells, on the other hand, express—in addition to the abnormally sized mutant mRNA—significant amounts of DdPYR5-6 mRNA (Fig. 5B).

These results suggest the possibility of using these *ura*$^+$ transformants to start a new round of 5-FOA selection, allowing subsequent introduction of different genetic elements into a single *D. discoideum* cells. Finally, these cells can be transformed with plasmids that confer G418 resistance, as G418 resistant strains can be transformed with pDU3B1-like plasmids followed by 5-FOA selection (Fig. 6).

General Applicability of 5-FOA Selection for *D. discoideum*. In addition to the described procedures that allow the identification of transformed and supertransformed *D. discoideum* cells, 5-FOA selection and *ura*$^-$ complementation offer a wide spectrum of sophisticated genetic analyses in *D. discoideum*.

1. It should be possible to guide genes of interest as single copies into the DdPYR5-6 locus if these genes are integrated, e.g., into the unique *Pvu*II site of the DdPYR5-6-containing fragment. For such a purpose, we constructed plasmid pUCPYR5-6, which carries the *Cla*I fragment from pDU3B1 blunt-end-ligated into a pUC-vector digested with *Pvu*II (Fig. 3B).
2. Because complementation of a *ura*$^-$ phenotype seems to require only one copy of an intact UMP-synthase gene, this gene can serve as selection marker in order to target other genes in a specific way. The *Cla*I cassette of pDU3B1 can be integrated into the coding region of any gene of interest, and this construct can be transformed into an *ura*$^-$ background. Based on *ura*$^+$ selection transformants with specifically disrupted or converted genes should be obtained with reasonable frequencies.
3. Extrachromosomal vectors can be constructed containing the UMP-synthase gene. Such vectors can be transformed into *ura*$^-$ cells, resulting in an *ura*$^+$ phenotype. Cells can be effectively cured of those plasmids if they are taken to a medium containing 5-FOA.
4. The UMP-synthase gene can be expressed under the control of an inducible promoter. Such constructs can serve as lethal reporters in *cis/trans* assays. Mutants can be selected that are affected in transactivating factors for the inducible promoter. Under conditions in which this promoter is normally transactivated, only those cells that carry a mutated transactivation factor can survive a 5-FOA selection.
5. Finally, 5-FOA resistance can be used as one dominant marker for the construction of diploids.

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REFERENCES

Alt FW, Kellems RE, Bertino JR, Schimke RT (1978): Selective multiplication of dihydrofolate reductase genes in methotrexate-resistant variants of cultured murine cells. J Biol Chem 253:1357–1370.

Boeke JD, Lacroute F, Fink GR (1984): A positive selection for mu-

tants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol Gen Genet 197:345–346.

Boy-Marcotte E, Jacquet M (1982): A *Dictyostelium discoideum* DNA fragment complements a *Saccharomyces cerevisiae ura*3 mutant. Gene 20:433–440.

Boy-Marcotte E, Vilaine F, Camonis J, Jacquet M (1984): A DNA sequence from *Dictyostelium discoideum* complements *ura*3 and *ura*5 mutations of *Saccharomyces cerevisiae*. Mol Gen Genet 193: 406–413.

Davies J, Smith DI (1978): Plasmid determined resistance to antimicrobial agents. Annu Rev Microbiol 32:469–518.

Dingermann T, Amon E, Williams KL, Welker DL (1987): Chromosomal mapping of tRNA genes from *Dictyostelium discoideum*. Mol Gen Genet 207:176–187.

Early AE, Williams JG (1987): Two vectors which facilitate gene manipulation and a simplified transformation procedure for *Dictyostelium discoideum*. Gene 59:99–106.

Franke J, Kessin R (1977):A defined minimal medium for axenic strains of *Dictyostelium discoideum*. Proc Natl Acad Sci USA 74: 2157–2161.

Jacquet M, Guilbaud R, Garreau H (1988): Sequence analysis of the DdPYR5-6 gene coding for UMP synthase in *Dictyostelium discoideum* and comparison with orotate phosphoribosyl transferases and OMP decarboxylases. Mol Gen Genet 311:441–445.

Knecht DA, Cohen SM, Loomis WL, Lodish HF (1986): Developmental regulation of *Dictyostelium discoideum* actin gene fusions carried on low-copy transformation vectors. Mol Cell Biol 6:3973–3157.

Mulligan RC, Berg P (1981): Selection for animal cells that express the *Escherichia coli* gene coding for xanthine-guanine phosphoribosyl transferase. Proc Natl Acad Sci USA 78:2072–2076.

Nellen W, Silan C, Firtel RA (1984): DNA-mediated transformation in *Dictyostelium discoideum:* Regulated expression of an actin gene fusion. Mol Cell Biol 4:2890–2898.

Nellen W, Firtel RA (1985): High copy number transformants and co-transformation in *Dictyostelium*. Gene 39:155–163.

Nellen W, Silan C, Saur U, Firtel RA (1986): Regulatory sequences in the promoter of the *Dictyostelium* actin 6 gene. EMBO J 5:3367–3372.

Rahmsdorf HJ (1977): A defined synthetic growth medium for *Dictyostelium discoideum* strain Ax-2. Hoppe Seyler's Z Physiol Chem 35:527–529.

Shaw W (1967): The enzymatic acetylation of chloramphenicol by extracts of R-factor-resistant *Escherichia coli*. J Biol Chem 242:687–693.

Watts DJ, Ashworth JM (1970): Growth of myxamoebae of the cellular slime mould *Dictyostelium discoideum* in axenic culture. Biochem J 119:171–174.

Welker DL, Williams KL (1982): A genetic map of *Dictyostelium discoideum* based on mitotic recombination. Genetics 102:691–710.

Wigler M, Pellicer A, Silverstein S, Azel R, Urlaub G, Chasin L (1979): DNA-mediated transfer of the adenine phosphoribosyl transferase locus into mammalian cells. Proc Natl Acad Sci USA 76:1373–1376.

Wilkie NM, Clements JB, Boll W, Mantei N, Lonsdale D, Weissmann C (1979): Hybrid plasmids containing an active thymidine kinase gene of herpes simplex virus I. Nucleic Acids Res 7:859–877.