Genetic Transfer in Plants Through Interspecific Protoplast Fusion

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Summary. Protoplasts of sexually incompatible species have been fused and in some combinations have given rise to somatic hybrid plants. Partial elimination of parental chromosomes from either species is common in such hybrids, but total chromosome loss has generally occurred only with phylogenetically unrelated pairings. Genetic function of one parent may be retained despite a complete loss of its chromosomes, suggesting that genetic introgression is possible in the absence of complete donor chromosomes. A model interspecific combination for such studies is the potato-tomato somatic hybrid for which numerous phenotypes and karyotypes are encountered at the outset, with a broader range observed in the second somatic generation.

A living plant cell consists of protoplasm bounded by a unit membrane (the plasmalemma) and encased in a cell wall of complex but largely cellulosic composition. As long ago as 1880 (*1*), the living component of the cell exclusive of the wall (the nucleus and cytoplasm) was termed the "protoplast." In 1910, Kuster (*2*) observed that protoplasts in a calcium salt solution would occasionally make contact and undergo a complete amalgamation of contents, but the process, called protoplast fusion, was infrequent and nonreproducibile.

With the development of efficient enzymatic methods for protoplast isolation in the late 1960's, ample quantities of protoplasts became available for fusion studies. Simultaneously, and beginning with the work of Takebe and his colleagues, isolated protoplasts of some plant species were cultured in defined media and induced to regenerate (from the Latin *regeneratus* meaning to bring into existence once again) complete plants (*3*).

Fusion of Protoplasts

Protoplast fusion begins with firm adhesion between the bounding membranes of adjacent protoplasts (Fig. 1A). As juncture points in the membrane barrier are dissolved, cytoplasmic constituents mix (Fig. 1, B and C). Eventually, the original two protoplasts round up into a sphere containing the nuclei of both parental cells (a dikaryon). A dikaryon is a homokaryocyte if the nuclei are identical and a heterokaryocyte if the nuclei are genetically different. Nuclei in the dikaryon may fuse before, during, or after mitosis and create a mononucleate hybrid cell or synkaryon.

Numerous chemical and physical conditions have been tested to induce protoplast fusion, but a major advance was the discovery of polyethylene glycol (PEG) as an efficient initiator. Polyethylene glycol [$HOCH_2$–(CH_2–O–CH_2)$_n$–CH_2OH] is highly soluble in water and ranges in molecular weight from less than 1000 to over 20,000. Schenk and Hildebrandt (*4*) first recognized the potential of PEG for stimulating protoplast adhesion, but the molecular weight of the PEG used, about 600, was too low for complete fusion. In 1974, Kao and Michayluk (*5*) and Wallin *et al.* (*6*) independently established the efficacy of PEG, in the molecular weight range of 1540 to 6000, for protoplast fusion. The compound has proved to be effective for protoplasts of all plant species tested and has similarly been applied to animal and bacterial cell fusions. A newer technique, electrofusion (*7*), also shows considerable promise. By this procedure, protoplast adhesion occurs in a nonuniform electrical field, and fusion of associated protoplasts is then induced by a short pulse of direct current. The technique is highly efficient and, unlike PEG, has relatively little immediate effect on protoplast viability.

Sexual Incompatibility and Chromosome Segregation

Sexual incompatibility precludes natural genetic exchange between distant or unrelated species; even within a single species there may be self- or cross-incompatibility. For related plants, fertilization may fail from cytoplasmic or nuclear factors (prezygotic incompatibility), or fertilized eggs may cease to develop at some early stage (postzygotic incompatibility). Postzygotic incompatibility can sometimes be overcome by culturing young embryos and stimulating them to develop into plants. Prezygotic incompatibility can occasionally be circumvented by pollinations in vitro (*8*). Unless otherwise specified, we use the term "sexual incompatibility" to refer only to those interspecific combinations for which no sexual means have yet created hybrid genotypes.

Extensive fusion research has been conducted on protoplasts of species that can either be hybridized sexually or crossed through some form of manipulation in vitro. Much of the research for these intra- and interspecific combinations has been reviewed (*9*). In this article, we discuss sexually incompatible pairings as a possible means of introducing new genetic information into a species.

During early studies, there was considerable hope for new amphiploid plants from fusions between protoplasts of sexually incompatible individuals. But, as had already been established in animal cell research, somatic combinations between distantly related or unrelated genomes was regularly followed by the elimination of parental chromosomes (chromosome segregation) from cell lines. The objectives of interspecific fusion have thus shifted away from synthesis of novel amphiploid plants toward the introduction of small genetic elements from alien species into ones of practical interest. Introgression of genes from diverse alien species could significantly expand germplasm pools for such characters as pest or stress resistance provided that the introduced genes were expressed and were capable of being manipulated by breeding techniques.

Protoplast fusion and mitosis are possible in heterokaryocytes regardless of the extent of relatedness (*10*), and fu-

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sions between protoplasts of distantly related plants do not necessarily result in the total elimination of chromosomes from either species. In a few cases, full chromosome sets are retained, whereas in others, modifications of chromosome structure permit synchronous duplication of alien chromosomes (or segments thereof) in the heterokaryocyte (*11*). However, developmental processes leading to the formation of embryos or shoot primordia are more sensitive to genetic constitution than mitosis is, and examples of plant regeneration from hybrid cells of sexually incompatible species are relatively scarce.

The most fully characterized interspecific combination is the "Arabidobrassica" hybrid created from fusions between two members of the Cruciferae family: *Arabidopsis thaliana* and *Brassica campestris*. Leaf protoplasts of *B. campestris* have 20 chromosomes ($2n = 2X = 20$) and callus cell protoplasts of *A. thaliana* are octaploid ($2n = 8X = 40$) (*12*). After fusion, some Arabidobrassica cell lines had 40 *Arabidopsis* chromosomes and 20 *Brassica* chromosomes, whereas two others had 40 chromosomes of each. Chromosomal rearrangements and interchanges were frequent in all lines. Later (*13*), Arabidobrassica plants were regenerated, but only from hybrid cell lines. No plants could be raised from unfused *A. thaliana* or *B. campestris* protoplasts. Plants were categorized as being either symmetric hybrids (true and stable somatic hybrids showing no evidence of chromosomal elimination) or asymmetric hybrids (where portions of either or both parental genomes have been eliminated). Asymmetric plants varied in chromosome number from 35 to 45, but individual karyotypes were stable. Structural modifications were evident in some chromosomes, and recombination was suggested in six of the hybrid plants. None was sexually fertile.

In another pairing between incompatible members of the same family, *Datura innoxia* and *Atropa belladonna*, only calluses with fleshy leaves were regenerated from synkaryons that retained all chromosomes of both parents (*14*). This developmental block was termed "somatic incompatibility," and, unlike Arabidobrassica, complete hybrid plants were only regenerated from hybrid cell lines that had lost one or more *A. belladonna* chromosomes (*15*).

The genus *Petunia* contains compatible and incompatible species. In early studies with sexually compatible individuals (*16*), somatic hybrid plants generally had the predicted amphidiploid chromosome number ($2n = 4X = 32$). Later (*17*), hybrid plants were obtained from fusions between *Petunia parodii* and *P. parviflora*, which are not sexually compatible. None was a true amphidiploid, but one cell line provided approximately 50 plants with a constant chromosome number of 31. The plants set pollen with a fertility quotient of 36 percent, compared with 98 to 99 percent fertility for parental pollen. Chromosome segregation was not observed in either regenerating cell lines or in hybrid plants.

In more distant interspecific combinations, there has been complete chromosome elimination for one parent. However, in a few examples, some genetic expression from the donor was retained despite total chromosome loss. Hybrid cell lines of *Petunia hybrida* fused with *Parthenocissus* and lacking chromosomes of the latter species expressed peroxidase isozyme patterns of both parents for at least 1 year (*18*). Dudits *et al.* (*19*) fused protoplasts of an albino nuclear mutant of carrot (*Daucus carota*) with those of chlorophyll-containing *Aegopodium podagraria*. Green plants from three callus lines had only carrot chromosomes, but molecular hybridization suggested integration of small *A. podagraria* chromosome segments. If so, the results resemble those in animal somatic cell fusions where, for example, genes from a chick have been incorporated into mouse cells in the absence of complete chick chromosomes (*20*).

It is evident that, except in very distant pairings, the extent or direction of chromosome segregation in interspecific hybrid cell lines is largely unpredictable, and for some combinations virtually any chromosome mix is possible. It is probable, however, that culture conditions more suited to one species than the other or relative stages in the mitotic cycle when protoplasts are isolated and fused might influence the direction or extent of chromosome segregation in proliferating cell lines. It may also prove advantageous to employ techniques such as x-irradiation or bromodeoxyuridine labeling of one parent to induce directional chromosome elimination, as originally described for Chinese hamster cells by Pontecorvo (*21*). Added control of chromosome segregation would be of use for reducing the number of potential genetic combinations that must be analyzed. Total loss of one set of chromosomes has already been achieved in *Nicotiana* fusions by lethal x-irradiation of one parent (*22*), but where chromosomal interchanges between species are desired, it may be advantageous to induce unidirectional chromosome loss over a series of mitotic cycles rather than strictly at the outset.

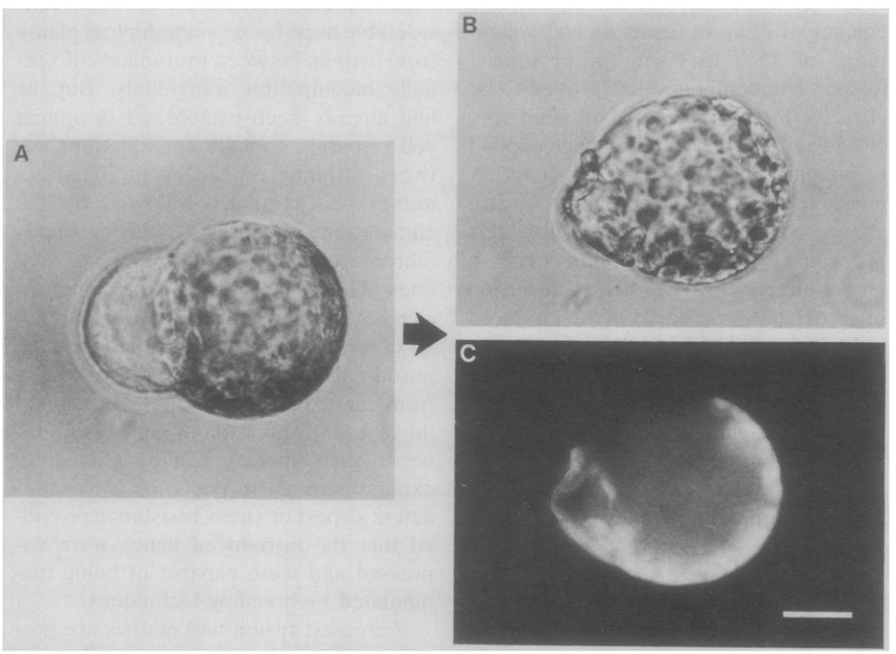

Fig. 1. Fusion of two plant protoplasts induced with polyethylene glycol. (A) The adhesion phase of a mesophyll protoplast from an albino protoclone of 'Russet Burbank' potato (on the left) and a larger leaf cell protoplast of tomato (on the right). (B) Nearly completed fusion between the same two protoplasts after a 15-minute incubation depicted under phase contrast microscopy. (C) The same protoplast pair as in (B) photographed under fluorescence microscopy. The potato protoplast had been stained with fluorescein isothiocyanate before fusion, while the tomato protoplast was left unstained. Transfer of the fluorescent dye to the tomato protoplast has occurred and the remaining juncture between the two protoplasts is clearly evident. Scale bar, 50 μm.

Gene Expression in Somatic Hybrid Plants

Proving the hybrid nature of regenerated plants requires a demonstration of genetic contributions from both parents. Morphological characters have often provided suggestive information, but the range of variability observed in plants raised from nonfused protoplasts (*23*) weakens the value of intermediate morphology as a sole criterion. Results are the most convincing when expression from both parents is in the form of identifiable biochemical markers that are encoded in plastid, mitochondrial, or nuclear DNA. Although relatively few biochemical markers have been analyzed in somatic hybrid plants, considerable differences in expression (or repression) do occur among individual hybrid lines from the same two parental species and even between different plants derived from a single hybrid cell line. Hence, interspecific protoplast fusions do not necessarily yield populations of somatic hybrid plants that manifest a uniform phenotype or that equally express designated molecular markers, even when all possess the predicted amphiploid chromosome number. Both nuclear and extranuclear gene expression may contribute to such differences because fusion produces hybrid cells that at least initially contain mixed organelle as well as mixed nuclear chromosome populations.

Extranuclear genes. Ribulose-1,5-bisphosphate carboxylase (RUDPcase) constitutes a major percentage of total protein in green plant tissues. The enzyme is composed of a chloroplast DNA–encoded large subunit and a nuclear DNA–encoded small subunit that exhibits Mendelian inheritance. Both subunits are composed of several discrete polypeptide chains. RUDPcase protein has routinely been studied in somatic hybrid plants as a marker for both nuclear and plastid genomes.

The RUDPcase large subunits from one parental species or the other, but not both, have regularly been observed in somatic hybrid plants of *Nicotiana* species (*24, 25*) and in potato-tomato hybrids (*26*). Rarely was plastid segregation unidirectional unless there was a genetic lesion in one plastid type or the application of selective pressure. Rather, the consensus is that after protoplast fusion, chloroplasts undergo a random sorting out that results in the survival of a single plastid type per cell (*27*).

Although intolerance of chloroplast mixtures is a consistent feature of individual cells, multiple plastid types do survive within the tissues of a regenerated plant (*28*). Iwai *et al.* (*29*) reported only the large RUDPcase subunit of *N. tabacum* in a *N. tabacum–N. rustica* somatic hybrid plant but later (*30*) found that in a population of nine androgenetic plants regenerated from anthers of the hybrid, two contained only the large subunit of *N. rustica*. Hence, plastids from both parents must have existed in the original plant.

Since chloroplast segregation predictably follows protoplast fusion, transfer of plastid-determined characters would be aided by techniques favoring the survival of the preferred plastid genome. Potential examples include resistance characters that are encoded in plastid DNA. Medgyesy *et al.* (*31*), for example, used streptomycin to select colony populations after fusions between mitotically inactivated (with iodoacetate) protoplasts of a streptomycin-resistant *N. tabacum* line and those of *N. sylvestris*. Both cybrid (cytoplasmic hybrid) and nuclear hybrid plants that expressed streptomycin resistance were obtained. Other plastid markers with in vitro selective potential include resistance to tentoxin (liberated by the fungus *Alternaria tenuis*) (*32*) and to triazine herbicides (*33*).

The fate of mitochondrial genomes in synkaryons and ultimately somatic hybrid plants is less clear. Belliard *et al.* (*24, 34*) regenerated hybrid plants from fusions between (sexually compatible) *N. tabacum* and a cytoplasmically male-sterile (cms) *N. debneyi*. Their results suggested retention of the male sterility character, possibly residing in mitochondrial DNA (mtDNA) in some hybrid plants, along with either coexistence of multiple mitochondrial types or recombination of mtDNA (*35*). Both phenomena are recognized in lower eukaryotes (*36*), but neither is proven for higher plants. Even so, additional circumstantial evidence is accumulating. Aviv and Galun (*32*) regenerated six classes of somatic hybrid plants from fusions between *N. sylvestris* and x-irradiated *N. tabacum* protoplasts. Of these, four were cybrid classes containing *N. sylvestris* nuclear genomes, and either (or both) of the chloroplast (tentoxin resistance) and cytoplasmic male fertility characters of *N. tabacum*. The degree of male fertility restoration was independent of plastid origin and hence was possibly correlated with mitochondrial composition. It is significant that in this instance male fertility was restored rather than eliminated through somatic fusions. Further evidence that a heteroplasmic (mixed cytoplasm) state for male sterility may be maintained for a considerable period comes from experiments with *Petunia* species. Izhar and Tabib (*37*) analyzed male-sterile somatic hybrids with the nuclear genome of *P. axillaris* and the cytoplasm of a male-sterile *P. hybrida* line. Two somatic hybrid plants displayed segregation of the sterility-fertility factors in the F_2 or F_3 generations following crosses with a cms tester line. Experiments were sufficient to reject mosaicism or acquisition of fertility-restoring nuclear genes as the explanation for segregation of the cms character in F_2 or subsequent generations.

Nuclear genes. Analysis of nuclear genome expression in putative somatic hybrid plants has essentially relied on morphological characters, isoenzyme distributions in polyacrylamide gels, or translation of specific genes. In the last category, the RUDPcase small subunit has been particularly useful. Small subunit polypeptide patterns produced after isoelectric focusing in a *p*H gradient are often definitive for a species, and even in the presence of a single plastid type, small subunit polypeptides of both parental species have been demonstrated (*32, 38*).

Somatic Hybrids Between Potato and Tomato

Potato (*Solanum tuberosum* L. spp. *tuberosum*) ($2n = 4X = 48$) and tomato (*Lycopersicon esculentum* Mill) ($2n = 2X = 24$) are members of the Solanaceae family but are not sexually compatible. In 1978, Melchers *et al.* (*39*) provided evidence for a somatic hybrid plant from fusions between protoplasts of a cultured dihaploid potato line and leaf cells of a chlorophyll-deficient tomato. Hybrid plants displayed morphological features of both parents, and analysis of the RUDPcase large subunit revealed that three plants carried the chloroplastic genome of tomato, whereas a fourth had that of potato. Those plants with a tomato plastome were termed "Tomoffeln" or "topatoes" while the ones possessing the plastome of potato were designated "Karmaten" or "pomatoes" (*40*). Subsequently (*26*), additional somatic hybrids were recovered; four topatoes and five pomatoes. None possessed the chromosome number of a true amphitetraploid ($2n = 4X = 48$), and it was not determined whether this was a consequence of chromosome segregation or of the use of mixoploid potato cells as protoplast donors. Some hybrid plants formed "tuber-like stolons" (but no tubers), and none set fertile flowers or fruit.

We have produced four somatic hybrid plants from fusions between chlorophyll-deficient protoplasts of a variegating protoclone (protoplast-derived clone) of the potato cultivar 'Russet Burbank' and the 'Rutgers' and 'Nova' cultivars of tomato. The potato protoclone (774) was previously described (*41*) and has a normal complement of 48 chromosomes. One somatic hybrid was identified from regenerated populations of the 774 potato crossed with 'Rutgers,' and three resulted from fusions of 774 and 'Nova.' The selection scheme developed for somatic hybrid colonies was based on the following observations. First, tomato mesophyll protoplasts divide in very low efficiency when cultured in the light at 24°C, whereas these conditions are optimal for potato mesophyll protoplasts. Second, small protoplast-derived calluses (p-calli) of potato cease growth when abscisic acid (ABA) is included in culture media at concentrations exceeding 0.5 milligram per liter. The growth rate of tomato p-calli, in contrast, is either unaffected or slightly stimulated at the same ABA levels. Tomato p-calli did not undergo shoot morphogenesis under conditions that were inductive for potato. When green adventitious shoots developed into small plantlets, the final screening characters, which appear when tomato shoots are regenerated from leaf disk callus, were employed; these characters were the formation of lobes and serrations in leaflets and reddish purple pigmentation in stems.

General morphological characters were consistent for all somatic hybrid plants. The basic plant growth habit was that of a potato-like vine; terminal and lateral leaflets were deep green in color and displayed serrations and lobing (Fig. 2, A and B); at 28°C, plants grew vigorously and anthocyanin (red) pigmentation accumulated in stems and on the underside of leaves; at 18° to 21°C, vegetative cuttings accumulated anthocyanins throughout and eventually died; white tubers (2 to 11 centimeters long) were produced that turned reddish purple if exposed to light during development; floral characters were identical to those of parental 'Russet Burbank' potato except for the 774–'Rutgers' hybrid whose petals were light yellow. Sterile fruit up to 2.5 cm in diameter having a yellow color at maturity and liberating a tomato-like odor developed on both 'Rutgers' and 'Nova' hybrids. All hybrids were sensitive to root-invading microorganisms and required initial establishment in sterilized vermiculite. Moreover, under routine greenhouse conditions, somatic hybrid plants were susceptible to the powdery mildew fungus, whereas neither the potato nor the tomato parents were susceptible. When taken together, these characters are found only in our somatic hybrids and were not reported for those previously described by Melchers (*26*), nor have they been observed in potato protoclonal populations.

Gel electrophoresis of restricted mtDNA and chloroplastic DNA (cpDNA) from 774–'Rutgers' and of 774–'Nova'-1 (*42*) revealed the extranuclear DNA's only of potato (Fig. 3, A and B), suggesting that the plants were true hybrids. If the plants had displayed mtDNA and cpDNA of both parents, they could have been chimeras composed of a mixture of potato and tomato cells rather than hybrids. Analysis of the small RUDPcase subunit from 774–'Rutgers,' 774–'Nova'-1 and 774–'Nova'-3 plants by isoelectric focusing in polyacrylamide gels (*43*) established the presence of small subunit polypeptides of both tomato and potato (Fig. 4). The 774–'Nova'-2 plant has not yet been examined. Profiles of several isozymes (peroxidase, malate dehydrogenase, esterase, 6-phosphoglucomutase, and polyphenol oxidase) were analyzed from leaf tissue of 774–'Rutgers' and compared with those of parental tomato and potato and with a random population of 15 regenerated protoclones of 'Russet Burbank' potato. For each enzyme, hybrid extracts shared specific bands with both potato and tomato. However, within the protoclonal population, individuals that also shared some tomato-specific bands for each enzyme were identified. No protoclone displayed all of the unique bands of the hybrid.

The potato-tomato somatic hybrid plants were cytologically examined at meiosis and mitosis (*44*). Observation of root tip cells of the 774–'Rutgers' hybrid shortly after initial transplanting consis-

Fig. 2. (A) Photograph of the 774–'Rutgers' somatic hybrid plant 2 months after transplanting. (B) Leaves of 'Russet Burbank' potato (on the left), 'Rutgers' tomato (middle), and the 774–'Rutgers' somatic hybrid (on the right).

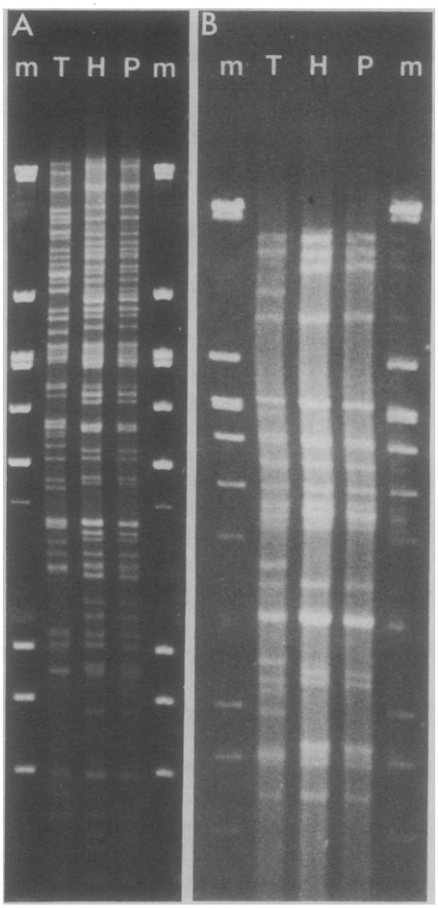

Fig. 3. Electrophoresis of organelle DNA's in 1 percent agarose gels. (A) Mitochondrial DNA's fragmented with the restriction enzyme Xho I. Lane T contains mtDNA from 'Rutgers' tomato, lane H from the 774–'Rutgers' hybrid, and lane P from the 774 potato protoclone. Lanes designated m are size marker fragments produced by independent digestions of lambda DNA by Eco RI and Hae III. (B) Chloroplast DNA's fragmented with Bam HI. Lanes are as in (A).

tently showed 72 chromosomes, the predicted number of a true amphiploid. Over the next 12 months, numerous vegetative cuttings were made from the hybrid, and root tip cells were analyzed for somatic chromosome number. The process was then repeated for each of the 774–'Nova' hybrids. Results from these experiments indicated that root tip cells of 'Nova' and 'Rutgers' somatic hybrid cuttings displayed chromosome numbers ranging from 62 to 72 depending on the cutting. The most frequently encountered chromosome number for the 'Rutgers' hybrid was 70, with greater variability observed for the 'Nova' hybrids. These data indicate a degree of mitotic instability and some chromosome segregation in vegetative cuttings, but not wholesale chromosome elimination. Phenotypic variations in the form of misshapen leaflets and color deviations were occasionally observed, particularly among cuttings of the 'Nova'-2 hybrid, but they could not be correlated with a specific change in chromosome number. Each of the hybrid plants flowered profusely but produced no viable pollen. Since parental 'Russet Burbank' potato expresses the same deficiency, it is uncertain whether some measure of fertility would be possible with another potato parent. In meiosis, there was clear evidence of chromosome elimination for 774–'Rutgers' and 774–'Nova'-1 (Fig. 5). The remaining two 'Nova' hybrids have not yet been analyzed.

One objective in our hybrid characterization is to determine whether karyotype stability can be achieved in populations of vegetative cuttings from hybrid plants. To this end, subpopulations from cuttings displaying chromosome numbers up to 72 are continually being made in order to establish whether any lines will stabilize. Moreover, protoplasts have been cultured from somatic hybrid plants of 774–'Rutgers' to determine whether karyotype stability will prevail in the second somatic (S_2) generation. To date, 50 plants have been regenerated from these protoplasts, and some are now being characterized. One S_2 protoclone displays the leaf morphology of potato, with no evidence of lobing or anthocyanin pigmentation. Electrophoretic analysis showed the RUDPcase small subunit of both tomato and potato. The predominant chromosome number of root tip cells was 64; hence, although the protoclone had assumed a more potato-like phenotype, it had probably not lost all tomato chromosomes. There are also S_2 protoclones that display a more tomato-like morphology than is seen in their hybrid parent, including more intense red pigmentation, more pointed terminal leaflets, and more extensive leaf serration. These and other S_2 protoclones are being analyzed for chromosome number to ascertain whether protoplast culture has conferred additional karyotypes to some members of the population and whether chromosome substitution or addition occurs. Evidence has accumulated that potato plants regenerated from mesophyll protoplasts display restructured chromosomes and translocations (*45*). If the same events take place in protoplasts of hybrid plants, translocations between potato and tomato chromosomes might also be anticipated.

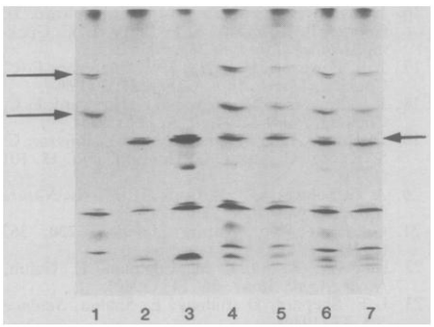

Fig. 4. Isoelectric focusing of small RUDPcase subunits in polyacrylamide gels. RUDPcase small subunit polypeptides from (lane 1) 774 potato protoclone, (lanes 2 and 3) 'Rutgers' tomato, (lane 4) 774–'Rutgers,' (lane 5) 774–'Nova'-1, and (lanes 6 and 7) 774–'Nova'-3. Two prominent potato-specific bands are identified by arrows on the left, and a tomato-specific band is identified by the arrow on the right.

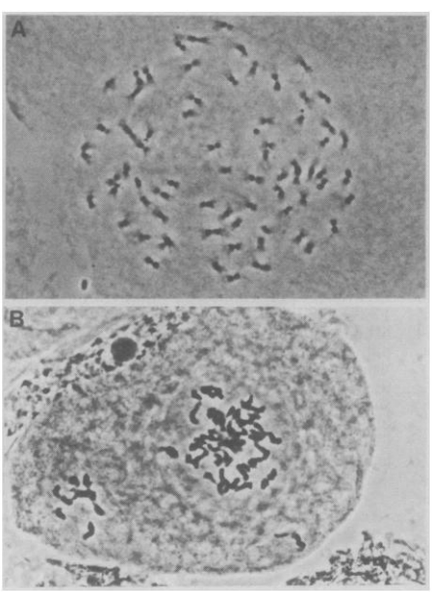

Fig. 5. (A) An early-metaphase mitotic figure in a root tip cell of the 774–'Rutgers' hybrid showing a complement of 72 chromosomes. (B) A pollen mother cell of the 774–'Rutgers' hybrid in metaphase I of meiosis in which chromosome elimination is under way. Estranged chromosomes are seen at lower left and lower middle.

Conclusions

In heterokaryocytes from such phylogenetically remote pairings as orange and tobacco protoplasts (*46*), *Nicotiana glauca* and soybean protoplasts (*47*), and *Haplopappus* protoplasts and human cells (*48*), either nuclei failed to fuse or synkaryons lost one set of chromosomes. Sometimes chromosome remnants have remained in cell lines through restructuring events, but there are no examples of modified chromosomes of an otherwise deleted genome persisting in a plant after fusion between members of different families. Such genetic incompatibility currently precludes transfer of whole chromosomes from a widely separated species to the regenerated plant. Despite total and unidirectional chromosome loss, it is still possible that in synkaryons proliferating in culture small chromosomal segments may be integrated into the surviving genome and thus enable genetic transfer.

In contrast to unrelated species, fusions between sexually incompatible members of the same family have produced hybrid plants that retained some chromosomes from both parents. These results are encouraging of potential somatic recombination (in its broadest sense) between formerly intractable species.

Interspecific protoplast fusions are a conceptual extension of interspecific sexual crosses to incompatible species. There is little control over what genetic information is retained and what is eliminated, and fusion lacks the potential precision of recombinant DNA methods. However, until the process of directed transformation with cloned genes reaches a higher level of sophistication, protoplast fusion offers a means for introducing genes from unconventional sources. Somatic hybrid plants such as the pomato are not of immediate value, just as is true of interspecific crosses between most distant sexually compatible species. In the latter, considerable backcrossing is needed to eliminate unwanted portions of the alien genome. Novel somatic hybrid plants are thus only the starting point of a genetic introgression scheme. If the hybrid plants are sexually compatible with either parent, removal of nondesirable material can be

straightforward. Where this is not true, protoplast regeneration from interspecific hybrids that undergo continuous chromosome segregation should provide novel genomic mixes. Moreover, since evidence is accumulating, at least in potato, that chromosome translocations are frequent in plants regenerated from mesophyll protoplasts, the coexistence of genomic sets may also allow translocation of, for example, tomato chromosome segments into potato chromosomes. Hence, unidirectional chromosome segregation combined with translocation or substitution could allow recovery of one parental phenotype with minor contributions from the other.

In a more immediate sense, protoplast fusion offers the opportunity of creating hybrid plants between related but sexually incompatible species. For example, commerical potato cultivars cannot be crossed with many related *Solanum* species without first passing through a bridging species such as *S. megistacrolobum*. Since many primitive *Solanum* species possess broad-spectrum resistance to disease (for example, *S. etuberosa* for resistance to leaf roll virus), protoplast fusion might allow a rapid introduction of resistance genes into potato germplasm pools.

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42. Mitochondrial DNA was extracted from leaf mitochondria of tomato cultivars 'Nova' and 'Rutgers,' from yellow and green leaf tissues of the variegating 774 potato protoclone, and from somatic hybrid plants according to the methods of R. Kemble *et al.* [*Genetics* **95**, 451 (1980)]. Chloroplasts were isolated from 3 to 10 grams of leaves from the plants listed above after 65 to 70 hours of incubation in the dark. Leaves were homogenized in 5 volumes of buffer A per gram of tissue (fresh weight) [R. Kolodner and K. K. Tewari, *Biochim. Biophys. Acta* **402**, 372 (1975)] with two 5-second bursts in a blender. The homogenate was filtered through two layers of cheesecloth and four layers of Miracloth before centrifugation at 2000*g* for 1 minute. Pellets were suspended in buffer A, centrifuged again, and suspended again in buffer A. Magnesium chloride (final concentration of 10 m*M*) and deoxyribonuclease (final concentration, 10 micrograms per gram of fresh weight) were added and the mixture was incubated at 4°C for 1 hour. Chloroplasts were centrifuged through a layer of buffer B (Kolodner and Tewari, *ibid.*) at 6000*g* for 20 minutes, washed twice in the same buffer, and lysed in 50 m*M* tris-HCl (*p*H 8.0), 10 m*M* EDTA, 2 percent Sarkosyl and 0.012 percent autodigested pronase at 37°C for 1 hour. Chloroplast DNA was extracted as described for mtDNA. Both mtDNA and cpDNA were fragmented with restriction endonucleases and subjected to electrophoresis in 1 percent agarose gels (according to the methods of Kemble *et al.*, cited above).
43. Analysis of RUDPcase protein in polyacrylamide gels followed the methods of D. Cammaerts and M. Jacobs [*Anal. Biochem.* **109**, 317 (1980)]. Briefly, 500 milligrams of leaf tissue from each parent and somatic hybrid were homogenized in 5 milliliters of extraction buffer [50 m*M* tris-HCl, 100 m*M* NaCl, 1 m*M* Na_2EDTA, 10 m*M* β-mercaptoethanol, and 1 percent (weight to volume) aprotinin (*p*H 7.5)] and centrifuged at 23,000*g* for 10 minutes. Precipitates obtained from supernatants at between 30 and 50 percent $(NH_4)_2SO_4$ saturation were resuspended in 300 microliters of 0.06*M* trisphosphate (*p*H 6.9) and 10 percent glycerol. These samples were subjected to electrophoresis in nondenaturing 4.5 percent polyacrylamide gels, RUDPcase bands were identified and cut out, and slices were equilibrated in 8*M* urea and 4 percent ampholytes [Sepralytes (*p*H 5 to 8); Separation Sciences, Inc., Attleboro, Mass.]. Isoelectric focusing to separate small RUDPcase subunits was done by placing equilibrated gel slices near the cathode on slabs, 0.5 millimeter thick, of polyacrylamide consisting of 6.25 percent acrylamide, 0.175 percent *N,N'*-methylenebisacrylamide, 8*M* urea, 2.6 percent Sepralytes (*p*H 5 to 8) and 1.4 percent Sepralytes (*p*H 3 to 10). Electrophoresis time for the gels (11 by 22 centimeters) was 5 hours at 10°C and 15 watts constant power. Gels were fixed for 30 minutes in a solution of 12 percent trichloroacetic acid, 4 percent 5′-sulfosalicylic acid, and 25 percent methanol and then washed for 30 minutes in 25 percent ethanol plus 10 percent acetic acid. Gels were stained for 2 hours in 0.1 percent Coomassie brilliant blue R-250 in 25 percent ethanol and 10 percent acetic acid.
44. Somatic chromosome numbers were established with root tip cells stained with 0.7 percent acetocarmine. Ten vegetative cuttings from each of two 774-'Rutgers' plants and five cuttings each from 774-'Nova'-1, 774-'Nova'-2, and 774-'Nova'-3 were analyzed.
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