A Transgene-Induced Mitotic Arrest Mutation in the Mouse Allelic With *Oligosyndactylism*

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Manuscript received June 18, 1996
Accepted for publication September 3, 1996

ABSTRACT

Oligosyndactylism (*Os*) is a radiation-induced mutation on mouse chromosome *8* associated with early postimplantation lethality in homozygotes and abnormal development of the limbs and kidneys in heterozygotes. The recessive lethal effect of *Os* is due to a mitotic block of the embryonic cells that becomes apparent at the blastocyst stage, but it is not known if the heterozygous effect of *Os* is due to haploinsufficiency of the gene responsible for the mitotic arrest, or is due to mutation(s) of other gene(s). We have recently described a transgene-induced recessive mutation, 94-A/K, that results in early postimplantation death of the embryos, and we have mapped this mutation to the same region of chromosome *8* where *Os* has been assigned. On the basis of complementation tests between transgenic and *Os*/+ mice, *in vitro* growth characteristics and increased mitotic index of 94-A/K embryos, and molecular structural analysis of 94-A and 94-K transgenic and *Os*/+ mice, we conclude that the 94-A/K mutation represents a new allele of *Os*. This insertional mutation should facilitate the isolation of a mammalian gene essential for normal progression of the cell cycle beyond metaphase.

KNOWLEDGE of the molecular mechanisms underlying the cell division cycle is important for understanding both normal and deregulated cell proliferation. Genetic analysis, mostly in lower eukaryotes, has contributed significantly to our current understanding of the components of the cell division machinery and of cell cycle control (McINTOSH and KOONCE 1989; MURRAY 1995a). Fewer mutants that affect cell cycle progression are known in mammalian cells, however, most of them have been identified through their effects on cells cultured *in vitro* or through their altered response to DNA damage and involvement in tumorigenesis (McINTOSH and KOONCE 1989; HARTWELL and KASTAN 1994; MURRAY 1995a). One developmental mouse mutation that is known to affect the progression through the cell cycle is *Oligosyndactylism*. *Os* is a radiation-induced mutation that shows an early, recessive lethal phenotype in homozygotes (VAN VALEN 1966) and dominant developmental defects in heterozygotes. In heterozygotes *Os* causes fusion of the second and third digits on all four limbs (GRÜNEBERG 1956, 1961), abnormal tendon attachments (KADAM 1962), a strain-dependent reduction in kidney size, and diabetes insipidus (FALCONER 1964; STEWART and STEWART 1969). Homozygous *Os* embryos die soon after implantation (VAN VALEN 1969). The lethal effect of *Os* is due to the inability of the mutant embryonic cells to complete mitosis, with progressive accumulation and subsequent degeneration of cells arrested in metaphase, beginning on day 4 of gestation (the blastocyst stage) (PATERSON 1979; MAGNUSON and EPSTEIN 1984). The mutant cells may survive to this stage because they utilize a stable maternal product supplied by the egg, or because the function of *Os* may not be required in the preblastocyst stage embryo (YEE *et al.* 1987). The defect in *Os*/*Os* embryos is cell autonomous, since the mutant cells cannot be rescued in aggregation chimeras (YEE *et al.* 1987). It is not known if the heterozygous and the homozygous effects of *Os* are due to defects in the same or different genes.

We have recently described a transgene-induced recessive lethal mutation associated with early postimplantation lethality in two transgenic lines that carry extra copies of the mouse phosphoglycerate kinase 1 (*Pgk1*) gene (PRAVTCHEVA *et al.* 1991; PRAVTCHEVA and WISE 1995). These lines, designated 94-A and 94-K, were derived from a common founder, but differ in the number of tandem transgene repeats and in the position of the transgene-cellular DNA junctions. Molecular analysis of the 94-A and 94-K insertion sites indicated that the transgenes are associated with overlapping but nonidentical deletions of endogenous DNA, possibly as a result of rearrangement of the transgene locus very early in the development of the founder (PRAVTCHEVA and WISE 1995). Mice with two copies of the transgene (A/A, A/K, or K/K) die between implantation and day 7 (we will use the designation 94-A/K for transgenic mice that contain any combination of the two transgene forms). The limbs of heterozygous 94-A and 94-K mice are normal and there are no obvious differences in kidney size between transgenic and nontransgenic mice, although precise measurements have not been

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carried out. Mapping of probes from the 94-A and 94-K transgene flanks with a BSS mouse backcross panel (from the JACKSON Laboratory) placed the transgene insertion in the subcentral region of mouse chromosome *8* (PRAVTCHEVA and WISE 1995). The assignment of *Os* to the same chromosome region (CECI 1994), and the death of 94-A/K and *Os/Os* embryos at approximately the same embryonic age, prompted us to investigate the relationship between the 94-A/K mutation and *Os*. The results of this investigation indicate that the 94-A/K insertional mutation is a new allele of *Os*.

MATERIALS AND METHODS

Mice: The production of the 94-A and 94-K transgenic mice has been described (PRAVTCHEVA *et al.* 1991). These mice contain several copies of a complete genomic clone of the mouse *Pgk1* gene. Although originally the transgene was placed on a mixed genetic background (CD1, B6D2F1), the transgenic lines have been maintained by backcrossing to FVB/N mice for >4 years. ROP/GnLe mice were obtained from the JACKSON Laboratory. They segregate the dominant (semidominant) mutations *Ragged* (*Ra*), *Os* and *Pintail* (*Pt*). All *Os*/+ mice that were used in the crosses with 94-A mice were wild type (wt) with respect to *Ra* and *Pt*.

DNA analysis: DNA was isolated from mouse tails using described procedures (SAMBROOK *et al.* 1989; HOGAN *et al.* 1994). Restriction digests were performed under conditions recommended by the manufacturer. Southern analysis was performed using standard techniques (SAMBROOK *et al.* 1989). Genotyping of mice with respect to their transgenic status was carried out with a probe from the 3′ end of the *Pgk1* transgene, which detects junction fragments after *Eco*RI digests in both 94-A and 94-K mice (probe C in Figure 1 and Figure 2B in PRAVTCHEVA and WISE 1995). Since the 5′ genomic flank of the 94-K transgene included in phage 5′K-3-3-4 contains highly repeated sequences, the probe from this phage (Figure 4D, this report) that detects a novel fragment in *Os* mice was preannealed with mouse repetitive (Cot-1) DNA before hybridization, under conditions recommended by the supplier (GIBCO/BRL). DNA from inbred strains 129/SvJ, A/HeJ, AKR/J, BALB/cJ, C3H/HeJ, CBA/J and SJL/J was received from the JACKSON Laboratory. DNA from mouse strain 101/Rl was a gift from Dr. LIANE B. RUSSELL.

Culture of embryos: Blastocyst-stage embryos were flushed from the uteri on day 4 of gestation (day 1 = day of plug) in Dulbecco's medium containing 15% fetal calf serum (D-FCS). The embryos were treated for 15 min at room temperature with M2 medium (HOGAN *et al.* 1994) containing 0.5% pronase and 0.5% polyvinylpyrrolidone (PVP), followed by three rinses in M2 medium containing 0.5% PVP. The treatment with pronase did not always result in the removal of the zona pellucida, but nevertheless facilitated hatching. The blastocysts were placed in individual drops of D-FCS under oil and cultured at 37° in a CO_2 atmosphere for 5 additional days. The drops were numbered and the embryos were photographed with an inverted phase contrast microscope on each consecutive day. This provided a record of the development of each individual embryo and allowed retrospective identification of homozygous transgenic embryos before the manifestation of their mutant phenotype.

Cytologic preparations and mitotic indices of early embryos: These were performed as described (TARKOWSKI 1966; MAGNUSON and EPSTEIN 1984) with small modifications. Blastocysts were removed on day 4 as described in the previous paragraph, and cultured in individual drops of medium (D-FCS) under oil for 2 additional days. On the second day of *in vitro* culture they were removed from the medium with a fine drawn pipet and transferred to individual wells of a 24-well Corning dish containing 1 ml of 0.9% sodium citrate on ice. This and the subsequent manipulations were monitored on a dissecting microscope. After a hypotonic treatment of 20–50 min each individual embryo was transferred with a fine pipet to a separate precleaned glass slide in a volume of hypotonic solution of <5 μl. The embryos were fixed on the slide by adding three 20-μl drops of methanol/acetic acid (7:3). After the fixative had spread on the slide, but before it had dried, a small drop of an acetic acid/lactic acid (80% syrup)/water mixture (5:1:4) was placed on top of the embryo with a fine pipet. The dissociation of the embryo cells was monitored on the microscope and was aided by dragging the acetic acid/lactic acid/water drop on the surface of the slide with the tip of the pipet. The slides were air-dried and stained with a 10% Giemsa solution for 10 min. Mitotic indices were determined by counting cells in mitosis and dividing the number by the total number of cells.

Structural analysis of the 94-A and 94-K insertion sites: The production of the genomic libraries from 94-A and 94-K mice and from mice that do not carry either transgene has been described (PRAVTCHEVA and WISE 1995). Phage isolation and restriction mapping were carried out by standard techniques (SAMBROOK *et al.* 1989). Phage 5′K-3-3-4, which includes the 5′ junction fragment of the 94-K transgene, was isolated with a probe from the *Pgk1* transgene (probe B in Figure 1 of PRAVTCHEVA and WISE 1995). Phage 27-1 and 43-2-1 were isolated in a chromosome walk initiated from the 3′ flank of the 94-K transgene. Fragments devoid of repeats, used as probes for hybridization with genomic or phage DNA, were identified by hybridizing phage or plasmid digests with total mouse genomic DNA.

RESULTS

Complementation analysis of *Os*/+ and 94-A mice: We crossed 94-A transgenic mice with *Os*/+ mice of the ROP/GnLe strain (from the JACKSON laboratory) or with *Os*/+ mice generated in the laboratory from the crosses between 94-A and ROP *Os*/+ mice. ROP/GnLe mice segregate three unlinked dominant (or semidominant) mutations: *Ragged* on chromosome *2*, *Os* on chromosome *8*, and *Pintail* on chromosome *4*. The *Os*/+ mice used in our crosses carried wild-type alleles of *Ra* and *Pt*. If the *Os* and 94-A mutations affect different genes, they should be able to complement each other and *Os*/+ progeny that carry the 94-A transgene should be viable. The *Os* phenotype was scored by inspecting the digits of the progeny, and the presence of the transgene was detected by Southern blotting using probes that can distinguish the transgene from the endogenous *Pgk1* gene. The results of these crosses are shown in Table 1. None of the 100 mice we have scored for the *Os* phenotype and the presence of the 94-A transgene were simultaneously *Os* and transgenic. The ratios of the mice in the three groups (*Os*, nontransgenic; wt for *Os*, transgenic; wt for *Os*, nontransgenic) closely match the expected ratios of surviving progeny if the *Os*/transgenic class is embryonic lethal. The absence of live transgenic progeny with the *Os* phenotype indicates that 94-A is unable to complement the recessive lethal effect of *Os*.

TABLE 1

Genotypes of live progeny from crosses between 94-A and *Os*/+ mice

Total no. analyzed	A^+, +	A^-, *Os*	A^-, +	A^+, *Os*
100	33	33	34	0

A^+, mice that carry the A transgene; A^-, mice that do not carry the A transgene; *Os*, oligosyndactylous mice; +, mice with a wild-type *Oligosyndactylism* phenotype.

***In vitro* growth of embryos from 94-A × 94-K crosses:** We have also examined the *in vitro* growth characteristics of embryos from 94-A × 94-K crosses and compared them with the previously described pattern of development of *Os/Os* embryos *in vitro* (PATERSON 1979; MAGNUSON and EPSTEIN 1984). In view of the early postimplantation death of homozygous transgenic embryos it was reasonable to expect that abnormal growth/survival of the mutant embryos may be detected during short term culture that spans the lethal period *in vivo*. Blastocysts from crosses between 94-A and 94-K mice were removed on day 4 of gestation and cultured individually in drops of medium under oil for an additional 5 days. The drops were numbered and the embryos were photographed on consecutive days. Under these conditions normal embryos hatch, attach to the dish, form a single layer trophectoderm outgrowth, and a dome of inner cell mass (ICM) cells protruding into the medium (GONDA and HSU 1980). Both the trophectoderm and the ICM cells remain viable for the duration of the culture period. This pattern of growth was observed in 100% of the embryos from the control crosses (where only one, or neither, parent was transgenic), and in 70% of the embryos from the 94-A × 94-K crosses (Figure 1, A, C, E and G, and Table 2). The remaining 30% of the embryos from 94-A × 94-K crosses showed the developmental pattern illustrated in Figure 1, B, D, F and H. The main characteristic of this class of embryos is their inability to form the typical ICM growth. A single layer of round, loosely attached cells formed instead, and these cells rapidly degenerated and detached in the medium by day 7. The trophectoderm cells of these embryos appeared normal, but were always fewer in number than those of the embryos with the normally developing ICM. Because this growth pattern occurred in 1/4 of the embryos from 94-A × 94-K crosses (and was absent in the control crosses), we concluded that the abnormally growing embryos are homozygotes for the transgene induced mutation. The morphologic characteristics of the class of abnormal embryos in the 94-A × 94-K cross are similar to those previously described for *Os/Os* embryos (PATERSON 1979; MAGNUSON and EPSTEIN 1984) and are consistent with the findings of *Os/Os* embryos *in vivo* (PATERSON 1979), where preferential death of the ICM cells and survival of giant trophoblast cells scattered in the decidua has been observed on day 6.

Mitotic indices in 94-A × 94-K embryos cultured *in vitro*: While preferential impairment of inner cell mass growth and survival has been observed in other recessive lethal mutations, the accumulation of cells in mitosis is a unique characteristic of *Os* (VAN VALEN 1966; PATERSON 1979; MAGNUSON and EPSTEIN 1984). The rounding up of the ICM cells in 1/4 of the 94-A × 94-K embryos is what would be expected from cells in mitosis, but cannot by itself be considered proof of the mitotic state of these cells. To unambiguously distinguish mitotic and nonmitotic cells and determine the mitotic index of embryos from 94-A × 94-K crosses we made cytological preps (TARKOWSKI 1966; MAGNUSON and EPSTEIN 1984) from day 6 embryos explanted on day 4 (blastocyst stage) and cultured for 2 days *in vitro* (Figures 2 and 3). This time period was chosen because previous analysis had shown that the difference between the mitotic indices of normal and *Os/Os* cells becomes very pronounced by day 6 (PATERSON 1979; MAGNUSON and EPSTEIN 1984). One hundred percent of the embryos from the control crosses and 72% of the embryos from the 94-A × 94-K crosses had a mitotic index of <6% (Figures 2A and 3A). Twenty-eight percent of the embryos from the 94-A × 94-K cross had a mitotic index of 20–40% (mean 31%) (Figures 2B and 3B). In comparison, PATERSON (1979) reported a mean mitotic index of 39.6% for day 5 embryos sectioned *in vivo*, and a mean mitotic index of 34% for cytological preps of embryos removed from the mother on day 4.5. The mitotic indices of presumed 94-A/K embryos are intermediate between the values of 11–36% (mean 21%) for day 5 embryos and 29–51% (mean 38%) for day 6 embryos reported by MAGNUSON and EPSTEIN (1984). The slightly lower values in 94-A/K day 6 embryos may be due to a developmental delay under our culture conditions, or to strain differences in the amounts or longevity of a maternally contributed *Os* (or equivalent) product utilized by early embryos in their mitotic divisions. The finding of elevated mitotic indices clearly demonstrates that, like the *Os* mutation, the 94-A/K mutation is associated with mitotic arrest. It also indicates that the gene required for the normal progression of the cell cycle has been disrupted in both 94-A and 94-K mice. The sister chromatids had separated, *i.e.*, initiated anaphase (Figure 2C), while still remaining close to each other, in some of the mitotically arrested cells of the 94-A × 94-K cross embryos. This has been previously observed in *Os/Os* cells (MAGNUSON and EPSTEIN 1984). It is not known if sister chromatid separation in these mutant cells occurred at its normal time (and is thus unaffected by the mutation) or was delayed as a result of the absence of the *Os* (94-A/K) product.

Probes from the 94-K transgene flank detect a rearrangement in *Os*/+ mice: If the 94-A/K transgene insertion and *Os* have affected the same gene, genomic probes from the region that has been deleted, or the region neighboring the transgene insertion, should detect rearrangements or loss of the cognate fragment in

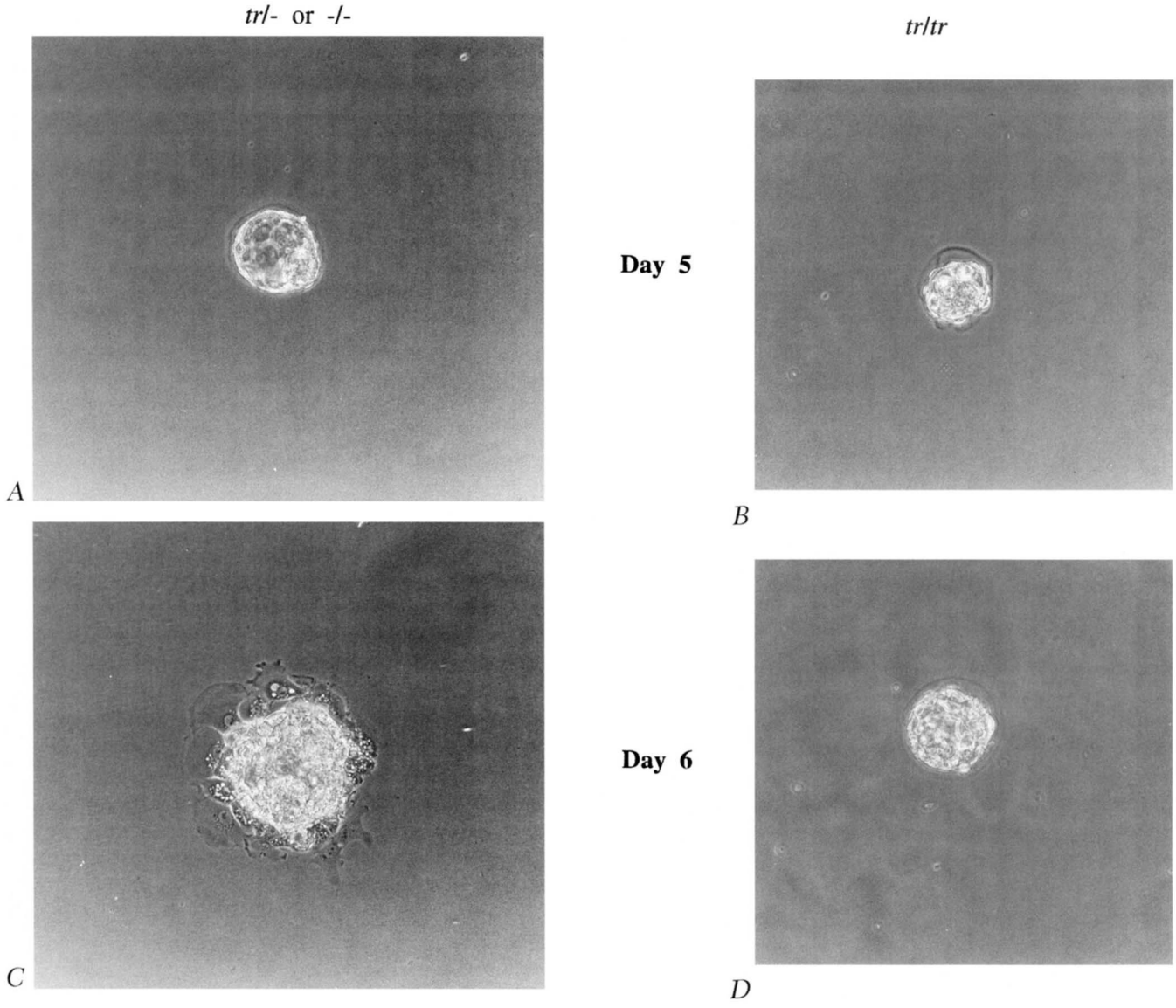

FIGURE 1.—*In vitro* culture of embryos from 94-A × 94-K and control crosses. Embryos were removed from the uteri on day 4 and cultured in individual drops of medium for five additional days. A, C, E and G illustrate the pattern of growth observed in all embryos from the control crosses (which contain one or no copies of the transgene, *i.e.*, *tr*/− or −/−) and in 70% of the embryos from the 94-A × 94-K crosses (presumed to be nontransgenic or heterozygous for the transgene), on days 5–8. B, D, F and H illustrate the abnormal pattern of growth seen in 30% of the embryos from the 94-A × 94-K crosses (presumed to be of the A/K genotype, *i.e.*, *tr/tr*) on days 5–8. Note the absence of proper ICM development and its replacement by loose round cells on top of the trophectoderm cells. The smaller number of the trophectoderm cells in the *tr/tr* embryo is also apparent. This *tr/tr* embryo also showed delayed hatching, which is a frequent but not obligatory characteristic of the embryos in this class.

Os/+ mice. That this is indeed the case is illustrated in Figure 4, A–C. A summary of the structural analysis of the 94-A and 94-K transgene loci is shown in Figure 4D. Both the 94-A and 94-K transgene insertions are associated with deletions (at least 26 kb are deleted in 94-K mice, and at least 48 kb in 94-A mice); the full extent of the deletions has not been determined yet. A probe from the 5′ genomic flank of the 94-K transgene (see phage 5′K-3-3-4 in 4D), which detects a novel (junction) fragment of ~11 kb in 94-K mice, also detects a novel fragment of ~13 kb segregating with the *Os* mutation in ROP/GnLe mice, or in crosses between *Os*/+ and 94-A mice (Figure 4A). The wild-type form of this fragment is polymorphic: it has a size of ~8 kb in most laboratory strains we have analyzed, including C57BL/6, DBA/2, ROP/GnLe, A/HeJ, AKR/J, BALB/cJ, C3H/HeJ, CBA/J, 101/Rl (Figure 4) and CD1 and 129/SvJ mice (not shown), whereas a high molecular weight fragment (>13 kb) is found in FVB/N mice, and a smaller fragment of ~3.5 kb is detected in SJL/J mice. The *Os* mutation was originally found in the progeny of an irradiated (101 × C3H)F1 male (GRÜNEBERG 1956). Both of these strains carry the common ~8-kb fragment (Figure 4C). The same probe detects

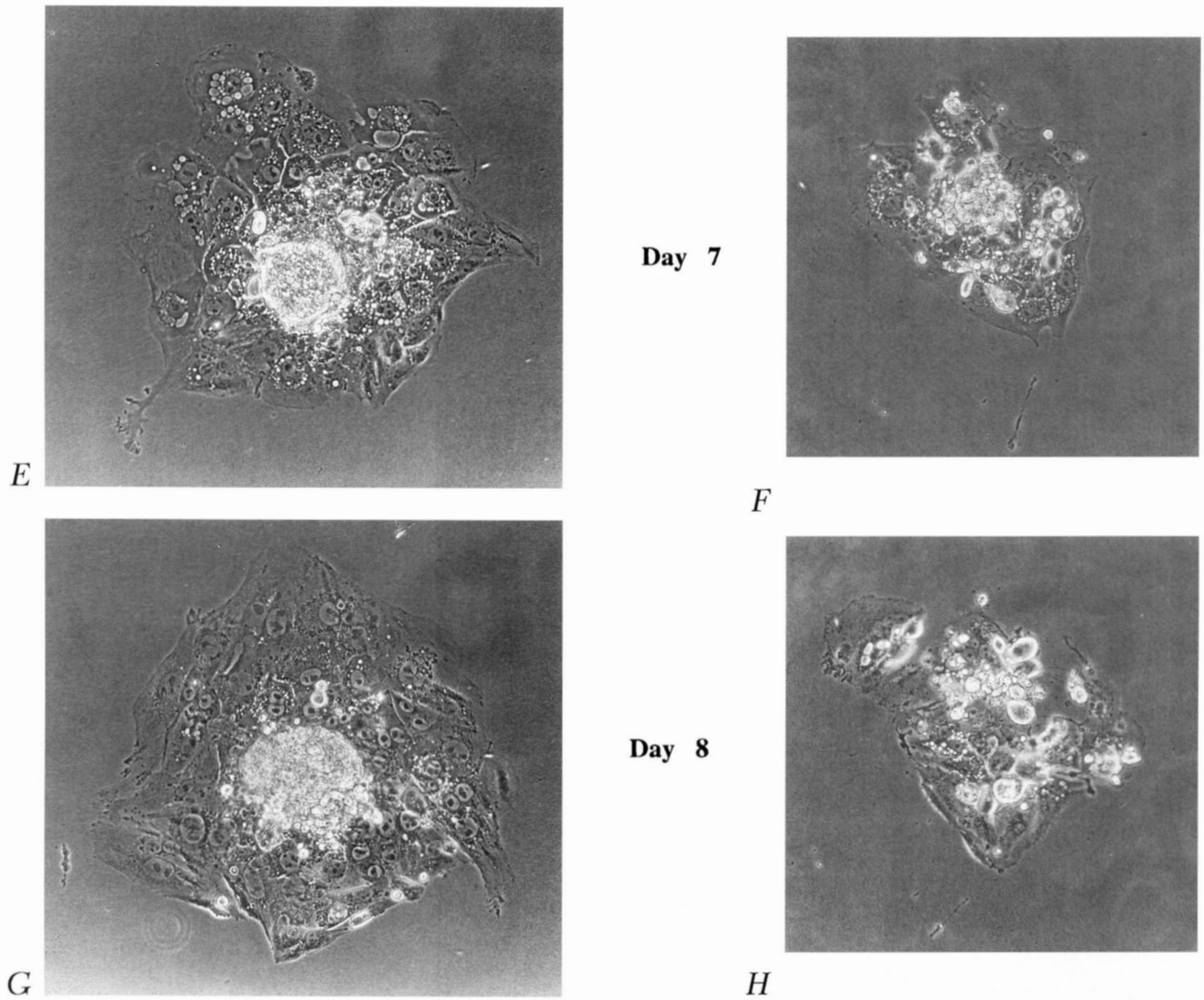

FIGURE 1.—*Continued*

a deletion in 94-A mice [compare the intensity of the largest fragment in the first two lanes (FVB/N) and the last two lanes (94-A) in Figure 4B]. In addition, transgenic progeny from crosses between ROP/GnLe Os/+ mice and 94-A mice have only the lower (ROP) molecular weight variant of the normal fragment and lack the larger FVB/N fragment (Figure 4C), indicating that the 94-A transgene carrying chromosome does not contribute any fragments hybridizing with this probe. These findings establish in molecular terms that the 94-A, 94-K and *Os* mutations are related. The probe from phage 43-2-1 (Figure 4D) that detects a deletion in both 94-A and 94-K mice detected no rearrangement or deletion in *Os*/+ mice (not shown). We are currently investigating whether the rearrangement in *Os* mice represents a deletion in a region adjacent to the 94-K deletion, or a small inversion.

TABLE 2

***In vitro* growth of blastocysts from 94-A × 94-K and control crosses**

Cross	Total no. of embryos	Blastocysts with normal TE and ICM growth	Blastocysts whose ICM fails to grow
A/− × K/−	56	39 (70)	17 (30)
−/− × −/−	18	18 (100)	0 (0)
−/− × K/− or A/− × −/−	42	42 (100)	0 (0)

−/− indicates a nontransgenic parent. A/− and K/− are heterozygotes for the 94-A and 94-K transgenes, respectively. Numbers in parentheses represent percentages.

DISCUSSION

On the basis of the results reported here, namely, (1) the complementation tests between 94-A and *Os*/+ mice, (2) the similarities of the *in vitro* growth pattern of 94-A/K and *Os*/*Os* embryos, (3) the high mitotic index in 1/4 of the embryos from 94-A × 94-K crosses, and (4) the detection of a novel fragment in *Os*/+ mice

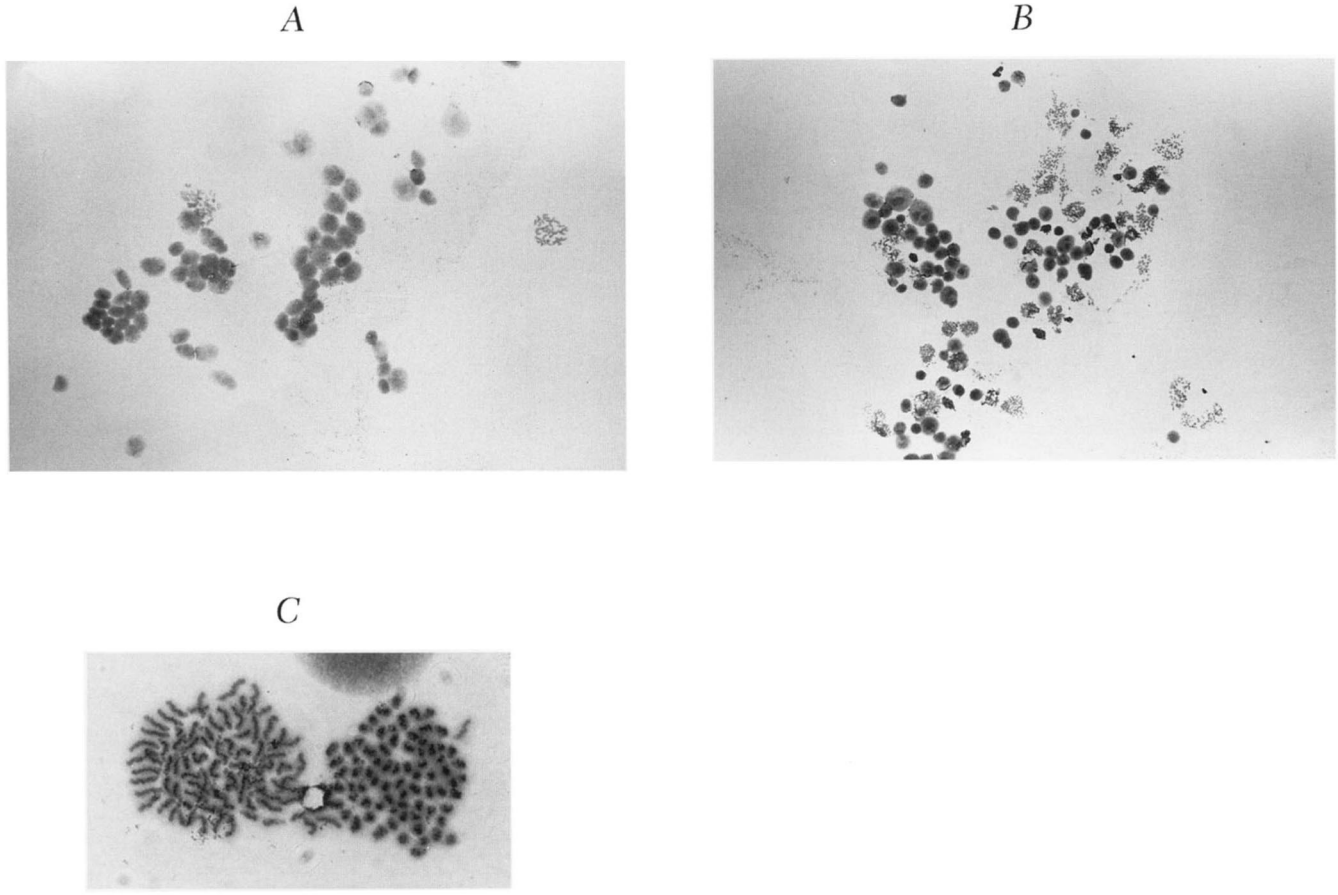

FIGURE 2.–Mitotic arrest in embryos from 94-A × 94-K crosses. Blastocysts were removed from the uteri on day 4 and were cultured *in vitro* for 2 additional days. Cytological preparations were made on the second day of *in vitro* culture. (A) Photograph of cells from a normal embryo. Two mitotic cells are visible in the field. (B) Photograph of cells from a presumed 94-A/K embryo. Multiple mitotic figures can be identified in the field; many of these mitotic cells have highly condensed, dot-like chromosomes. (C) Sister chromatid separation in two mitotic cells from the embryo shown in B.

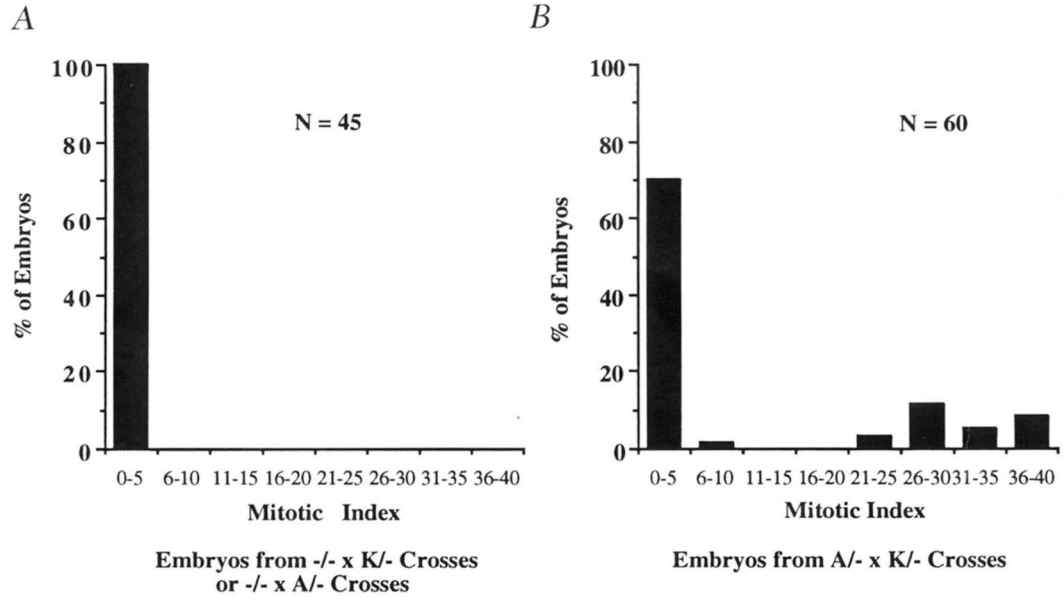

FIGURE 3.—Mitotic indices of embryos on day 6 of *in vitro* culture. (A) Embryos from control crosses between a nontransgenic and a transgenic (94-A or 94-K) parent. (B) Embryos from 94-A × 94-K crosses. N, number of embryos analyzed.

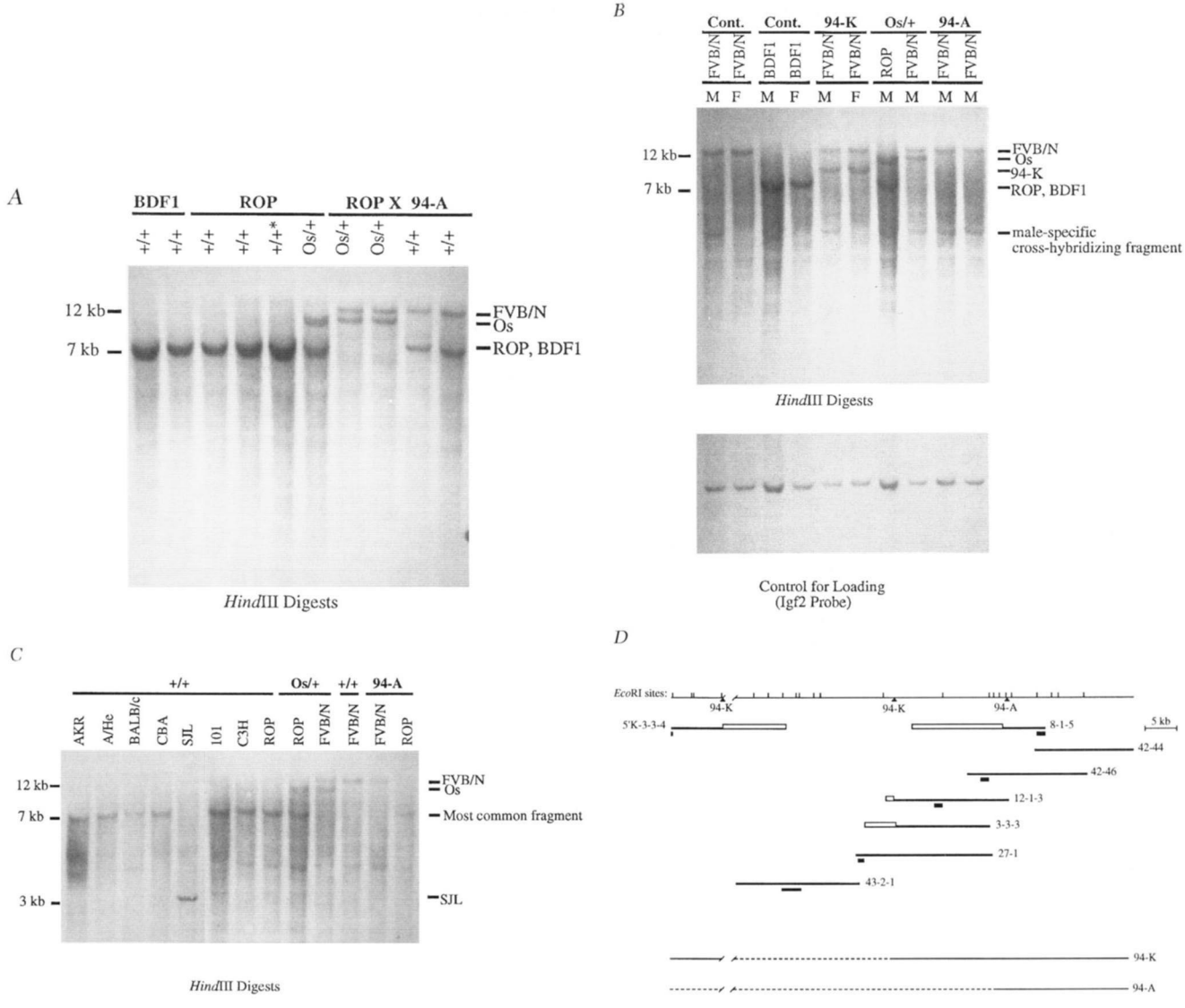

FIGURE 4.—A probe from the 5′ flank of the 94-K transgene detects a novel fragment in 94-K and *Os*/+ mice, and a deletion in 94-A mice. (A–C) *Hind*III digests, 0.5% agarose gel, probe is a *Bam*HI subclone from phage 5′K-3-3-4 (Figure 4D). (A) Detection of a novel fragment in *Os*/+ mice. +/+ indicates wt for *Os*. BDF1, (C57BL/6J × DBA/2)F1; ROP, ROP/GnLe. All ROP DNAs are from mice that are wt for *Ra*. The lane marked with an asterisk contains DNA from a +/*Pt* mouse. The remaining two ROP lanes are wt for *Pt*. The lanes marked ROP × 94-A contain DNA from nontransgenic progeny from crosses between ROP/GnLe *Os*/+ and 94-A mice. The polymorphic normal fragment recognized by the probe in FVB/N mice (>13 kb) and BDF1 or ROP mice (~8 kb) is indicated on the right. A novel fragment of ~13 kb (marked *Os* on the right) cosegregates with the *Os*/+ phenotype on both ROP/GnLe and FVB/N (94-A) background. The position of two molecular weight markers from the BRL 1-kb ladder is indicated on the left. (B) Comparison of the rearrangements detected with the 5′K-3-3-4 probe in 94-A, 94-K and *Os*/+ mice. Cont. indicates nontransgenic, wt for *Os*, control DNA. The strain designations on top of the individual lanes refer to the source of the normal allele of the *Os* (94-A/K) locus. M and F indicate male and female, respectively. The position of the normal fragments in FVB/N and BDF1 or ROP/GnLe mice, and the position of the the novel fragments detected in 94-K mice (~11 kb) and *Os*/+ mice is indicated on the right. The lower intensity of the FVB/N fragments in 94-A mice compared with nontransgenic FVB/N mice indicates that the corresponding fragment has been deleted in 94-A mice. The blot shown in B was stripped and rehybridized with a fragment of the unlinked *Igf2* gene as a control for loading. The 5′K-3-3-4 probe cross-hybridizes with a male-specific fragment whose detection is sensitive to small variations in the hybridization/washing conditions (compare A and B). Molecular weight markers are shown on the left. (C) Polymorphism of the fragment detected by the 5′K-3-3-4 probe among mouse laboratory strains, and absence of this fragment from the 94-A chromosome *8*. The strain origin of the DNA is indicated above each lane. +/+ indicates wild type for *Os*. In the *Os*/+ and 94-A lanes the strain designation refers to the origin of the normal (+) chromosome *8*. A variant fragment of ~3.5 kb is detected in SJL/J mice. The 94-A transgenic mouse from a cross between 94-A and ROP mice contains only the ROP fragment (94-A/ROP, last lane). (D) Structure of the genomic region at the 94-A and 94-K integration site. The top line represents the extent of the genomic region analyzed. Triangles below the line mark the points where the 94-A and 94-K transgenes are joined to endogenous sequences. Thick lines represent genomic sequences present in individual phage clones (5′K-3-3-4, 8-1-5, etc.). Black bars below the the phage indicate the position of probes that were used for walking, or for the hybridizations shown in A and B. The open rectangles in phage 8-1-5, 12-1-3, 3-3-3, and 5′K-3-3-4 represent the transgene portion of these phage. The extent of the deletions associated with the 94-A and 94-K transgenes are represented by the dotted lines at the bottom of the figure. The 94-A deletion is larger and encompasses the deletion associated with the 94-K transgene.

by a probe from the 5′ flank of the 94-K transgene, we conclude that the insertional mutation in 94-A/K mice has affected the same gene that is responsible for the mitotic arrest and early postimplantation lethality of *Os/Os* embryos. The preferential involvement of ICM cells in 94-A/K and *Os/Os* embryos would be attributed to the continuous mitotic activity of these cells and their need for the 94-A/K (*Os*) gene product, whereas trophectoderm cells, which undergo endoreduplication without mitosis, would be able to survive without it. The absence of limb abnormalities in the 94-A and 94-K mice is most easily explained by assuming that the radiation-induced *Os* mutation has affected more than one gene (*i.e.*, through a deletion or an inversion), and that the limb and kidney abnormalities and the postimplantation lethality are due to defects in different genes. Further molecular characterization of *Oligosyndactylism* will determine if this is the case.

Studies by MAGNUSON and EPSTEIN (1984) on the nature of the mitotic block in *Os/Os* cells have determined that these cells are unable to make the metaphase/anaphase transition (as indicated by the chromosome alignment on the metaphase plate). In normal cells metaphase is followed by disjoining of the sister chromatids and their movement toward the poles (anaphase A) and elongation of the mitotic spindle (anaphase B). The metaphase-anaphase transition represents one of the checkpoints in the cell cycle (HARTWELL and WEINERT 1989; HOYT *et al.* 1991; LI and MURRAY 1991; MURRAY 1992; WELLS 1996). Its function is to assess the formation of proper microtubule/kinetochore attachments and chromosome alignment, and to prevent progress in the cell cycle (and the ensuing aneuploidy) if improperly attached, misaligned, lagging chromosomes are detected in the cell (CAMPBELL and GORBSKY 1995; LI and NICKLAS 1995; RIEDER *et al.* 1995). A variety of factors that affect the dynamics of microtubule assembly/disassembly and interaction with the kinetochore may be able to activate this checkpoint and delay or block exit from metaphase. The mitotic spindle, centrosomes, and kinetochores are apparently normal in *Os/Os* cells (MAGNUSON and EPSTEIN 1984). Similarly, no abnormalities were detected in α and β tubulin, and the spindle microtubules showed normal sensitivity to cold temperatures and depolymerizing agents (MAGNUSON and EPSTEIN 1984). Thus obvious abnormalities in the structural components of the mitotic apparatus are unlikely to be the reason for the mitotic arrest of *Os/Os* cells. It is known, however, that drugs that induce mitotic arrest by affecting microtubule stability are able to block cells in mitosis even at concentrations that have little visible effect on the structure of the mitotic spindle (JORDAN *et al.* 1992). The existence of such subtle alterations in the components of the microtubule/kinetochore interaction in *Os/Os* (94-A/K) cells at present cannot be ruled out. It is therefore possible that the role of the *Os* (94-A/K) gene product may be in normal microtubule/kinetochore attachment and dynamics, and its absence may prevent the cells from passage through the metaphase/anaphase checkpoint.

Alternatively, the *Os/Os* (94-A/K) embryo cells may lack a product required to activate and carry out the complex series of events that are initiated upon successful passage through this checkpoint. A crucial event in this cell cycle transition is the degradation of cyclin B and the ensuing inactivation of the maturation-promoting factor, a complex of cyclin B with $p34^{cdc2}$ (NURSE 1990; KING *et al.* 1994). Cyclin B degradation is mediated by polyubiquitination and requires the normal function of a multiprotein complex (anaphase-promoting complex) that includes the products of the budding yeast genes CDC16, CDC23 and CDC27, or their homologues in other species (LAMB *et al.* 1994; IRNIGER *et al.* 1995; KING *et al.* 1995; MURRAY 1995b; TUGENDREICH *et al.* 1995). Activation of the spindle assembly checkpoint results in sustained high activity of MPF (HUNT *et al.* 1992; MINSHULL *et al.* 1994). In addition, species differences in the duration of the cell cycle block imposed by the spindle assembly checkpoint correlate with the ability to maintain high MPF activity (KUNG *et al.* 1990). Thus genetic defects that prevent the normal decline in activity of MPF would be expected to result in a mitotic block. However, maintaining high levels of cyclin B (and MPF activity) through overproduction of the protein, or through use of nondegradable cyclin, delays chromosome decondensation, nuclear membrane reconstitution, and cytokinesis, but has no effect on sister chromatid separation, which proceeds on an unaltered schedule to form two separate clusters at the opposite poles of the spindle (HOLLOWAY *et al.* 1993; SURANA *et al.* 1993; IRNIGER *et al.* 1995). Introducing nondegradable cyclin into human HeLa cells also did not prevent the movement of the chromosomes away from the metaphase plate (GALLANT and NIGG 1992). On the other hand, sister chromatid separation and anaphase movement were prevented by introducing an N-terminal fragment of cyclin B, which is believed to interfere with the degradation of an yet unknown protein that may act as an inhibitor of the normal process of sister chromatid separation (HOLLOWAY *et al.* 1993). At present we do not know if sister chromatid separation in *Os/Os* (94-A/K) embryos is delayed (suggesting a possible defect that slows down the degradation of the protein maintaining sister chromatid cohesion), or takes place at its normal time. The metaphase arrest of *Os/Os* embryo cells, however, suggests a mitotic block at an earlier stage than the mitotic block observed in the presence of high cyclin B levels and MPF activity.

The transition from metaphase to anaphase is accompanied by marked changes in the degree of phosphorylation of a large number of cellular proteins. The monoclonal antibody MPM2 recognizes a phosphorylated epitope shared by at least 16 different proteins located on the chromosomes or in the cytoplasm of mitotic

cells in a wide variety of species (DAVIS *et al.* 1983). In normal cells the MPM2 reactivity appears with entry into mitosis and is lost as cells exit from metaphase. Cells arrested in metaphase by Nocodazole (a treatment that results in microtubule depolymerization and activation of the metaphase-anaphase transition checkpoint) maintain their high level of MPM2 staining for as long as 18 hr (VANDRE and BORISY 1989). In this respect it is interesting to note that some mitotically arrested cells in *Os/Os* embryos have been found to exhibit no fluorescence (*i.e.*, to have a postmetaphase type staining) with the MPM2 antibody (HIRAOKA *et al.* 1989). This may point to some defect in the process of phosphorylation of the MPM2 epitope (which accompanies entry into mitosis) in *Os/Os* cells (HIRAOKA *et al.* 1989). Alternatively, the absence of MPM2 staining in some *Os/Os* mitotic cells may be the result of dephosphorylation that normally takes place at the metaphase/anaphase transition. In this case, the absence of staining in these cells will simply reflect a more advanced stage in the cell cycle that they have been able to reach in the absence of the *Os* (94-A/K) gene product. The separation of sister chromatids in fission yeast requires the action of phosphatases (OHKURA *et al.* 1989). The normal course of anaphase in *Aspergillus nidulans* (DOONAN and MORRIS 1989), Drosophila (MAYER-JAEKEL *et al.* 1993), and mammals (VANDRE and WILLS 1992) also requires the activity of protein phosphatases (PP). In *A. nidulans*, PP1 is required for the removal of the MPM2 epitope (DOONAN and MORRIS 1989). In this regard it would be interesting to determine if the cells in which the sister chromatids have already been separated (as in Figure 2C) are the ones that have lost the phosporylated epitope recognized by MPM2.

Studies on mitosis in frog egg extracts have led to the suggestion that poleward movement of sister chromatids is an automatic consequence of the loss of cohesion between the sister chromatids: no change in the force applied to the kinetochore has been noted at the transition from metaphase to anaphase (HOLLOWAY *et al.* 1993). If this is so in mammalian cells, the absence of anaphase movement of sister chromatids that have clearly been separated from each other in 94-A/K (Figure 2C) and in *Os/Os* embryos (MAGNUSON and EPSTEIN 1984) is unexplained. In normal cells this chromosome movement is initially mainly the result of depolymerization of the plus (kinetochore) end of the kinetochore fibers (microtubules connecting the kinetochore and the spindle pole) and appears to require the presence of motor proteins that can couple microtubule depolymerization and chromosome movement (MITCHISON *et al.* 1986; GORBSKY *et al.* 1987; DESAI and MITCHISON 1995). Separation of sister chromatids without poleward movement has been observed in some cells treated with microtubule depolymerizing agents (RIEDER and PALAZZO 1992), where it can be explained by the microtubule effects of these agents. The limited studies on the mitotic spindle in *Os/Os* embryos do not preclude the existence of subtle alterations that prevent the poleward separation of the sister chromatids. However, the observation of premature centromere division and sister chromatid separation without poleward movement as an age related, or inherited, cytogenetic abnormality in humans suggests the possibility that rather than being an automatic consequence of loss of sister chromatid cohesion, anaphase movement of sister chromatids is itself an independently regulated cell cycle event (FITZGERALD *et al.* 1975; RUDD *et al.* 1983). Elucidation of the molecular nature of the *Os* (94-A/K) mutation may bring about a better understanding of the consecutive steps in the metaphase/anaphase transition.

In summary, we have characterized an insertional mutation in transgenic mice and have determined that it represents a new allele of *Oligosyndactylism*. *Os* affects a cellular process that is essential for the normal transition of the cell division cycle from metaphase to anaphase. The insertional mutation will facilitate the isolation and characterization of the affected gene and will allow a better understanding of the sequence of molecular and cytologically observable events (dephosphorylation, protein degradation, sister chromatid separation) at the metaphase-anaphase transition.

We thank Dr. LIANE B. RUSSELL for the gift of mouse strain 101/RI DNA. We acknowledge the excellent technical assistance of SARA SEEMATTER. This work was supported by the Pediatric Research Institute of Cardinal Glennon Children's Hospital New Project Development Fund, and by a Fleur-de-Lis grant to D.P.

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Communicating editor: N. A. JENKINS