PREIMPLANTATION DEVELOPMENT OF t^{w32}/t^{w32} LETHAL MOUSE EMBRYOS IN VITRO*

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ABSTRACT

Previous work has shown that mouse embryos homozygous for the t^{w32} allele at the complex T or Brachyury locus die during the morula-to-blastocyst transformation (68-80 hours post coitum). Objectives of this study were to: (1) recover and culture preimplantation mouse embryos from δ+/t^{w32} x ♀+/t^{w32}, δ+/t^{w32} x ♀+/T, δT/t^{w32} x ♀T/t^{w32}, and δICR x ♀ICR matings from 54 to 96 hours post coitum (hpc), (2) compare developmental chronology between embryos of experimental and control crosses, (3) determine percentage of lethal mutants (t^{w32}/t^{w32}), and (4) discover new information pertinent to t^{w32} allele expression.

A total of 56 experimental and control females yielded 544 cleavage stage embryos which were cultured and observed over the 54-96 hpc interval. A comparison of developmental chronology indicated: (1) embryos from experimental crosses were more advanced than control embryos at 54-59 hpc, (2) no developmental stage differences between groups were apparent at 65-69 hpc, and (3) the presence of lethal mutants within experimental populations became evident at 66-74 hpc.

In experiment crosses (δ+/t^{w32} x ♀+/t^{w32} and δT/t^{w32} x ♀T/t^{w32}) 36.8% (82/223) and 41.5% (22/53) of the embryos underwent developmental arrest. Since losses in control crosses for those two sets of experiments accounted for 0.5% (1/194) and 1.4% (1/74) respectively, 36.3% and 40.1% of the experimental populations were presumed to be t^{w32}/t^{w32} lethal mutants.

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Cultural observations on lethal t^{w32} homozygotes reveal that mutants may never completely compact. Some presumed mutants produce blastocoelic spaces but fail to form well-defined blastocoels. Finally, in our experimental system the phenocritical period extends over the 66-79 hpc interval.

INTRODUCTION

Preimplantation mouse embryos homozygous for t^{12} and t^{w32} alleles at the complex T (Brachyury) locus undergo developmental arrest during the morula-to-blastocyst transformation. According to Bennett and Dunn (1964), t^{12} and t^{w32} alleles are the same; however, Hillman and Hillman (1975) stated ". . . the effects of these two alleles (t^{12} and t^{w32}) result in the same or in a closely associated developmental aberration."

Following extensive *in vitro* observations on control and t^{12}/t^{12} embryos Mintz (1964) reported that certain changes which normally occur prior to blastocyst formation are either limited or completely absent in t^{12} homozygotes. These normal events include: (1) decrease in cytoplasmic granularity (or increase in cytoplasmic translucency), (2) formation of numerous prominent spherical bodies, and (3) coalescence of spherical bodies to form blastocoelic cavities. Calarco and Brown (1968) using electron microscopy reported ". . . the most prominent cytoplasmic structure in normal and t^{12}/t^{12} morulae is spherical vesicles containing medium density material"; they also noted that mutant cells ". . . appeared more rounded and less closely applied . . ." than normal cells.

In vitro observations have revealed differences between t^{12} and t^{w32} alleles. The t^{12}/t^{12} lethality (phenolethal period) is expressed from 8-cell to early blastocyst stages, with most embryos arresting as late morulae (Hillman et al., 1970). The phenolethal period for t^{w32}/t^{w32} embryos is from 8-cell to late morula stages, with most embryos arresting as early morulae (Hillman and Hillman, 1975). Hillman et al. (1970) also reported that embryos arresting early (8-12 cell stage) are usually normally shaped (round); when embryos arrest later (early to mid-morula stage) blastomeres may be either round or misshapen. Although Bennett and Dunn (1964) could not distinguish t^{12} from t^{w32} homozygotes on the basis of embryo recovery and observation (with no culture), Hillman (1975) does indeed review data supporting the notion that t^{12} and t^{w28} are separate alleles.

Further information on generation of the lethal syndrome in t^{w32} homozygotes should be obtained by culturing and observing embryos from

control and experimental crosses. Objectives of the present study were to: (1) recover and culture mouse embryos from ♂T/t^{w32} x ♀T/t^{w32}, ♂ICR x ♀ICR, ♂+/t^{w32} x ♀+/t^{w32}, and ♂+/t^{w32} x ♀+/T crosses from 54 hours *post coitum* (hpc) to 96 hpc, (2) compare developmental chronology of experimentals and controls, (3) determine percentage of developmentally arrested morulae (t^{w32}/t^{w32}) in experimental versus control crosses, (4) describe the genesis of the t^{w32} homozygous lethality and (5) discover new information pertaining to t^{w32} gene expression.

METHODS

Experimental crosses were made between proven +/t^{w32} males and +/t^{w32} females and between tailless males and females (T/t^{w32} x T/t^{w32}); control crosses were ♂+/t^{w32} x ♀+/T and ICR x ICR, respectively. The developmental success of embryos from each of these four crosses is recorded in Tables 1 and 2. Embryos were recovered from experimental and control crosses at 54-59 hours post coitum (hpc) and cultured in Brinster's Medium BMOC-3 (GIBCO). Oviducts of T/t^{w32}, ICR, +/t^{w32}, and +/T females were flushed with BMOC-3 and cultured with 2.0 ml BMOC-3 in small Falcon plastic petri dishes in a gas flow incubator kept at 37° C and gassed with 5% CO_2 in air. Embryos were observed, scored for developmental success, and photographed at given intervals. Careful observations were made during each of the following time periods: 54-59, 60-64, 65-69, 75-79, 80-84, 85-89, and 90-94 hpc. Criteria used for staging embryos and assessing developmental progress are comprehensively outlined in Granholm and Brenner (1976) and Johnson (1976).

RESULTS

Differences in developmental rates between embryos of experimental (+/t^{w32} x +/t^{w32}) and control (♂+/t^{w32} x ♀+/T) matings were observed at the 54-59 hpc observation interval; 53.2% of the experimental group were

either partially or completely compacted versus 32.6% of the control group. At the 65-69 hpc interval, developmental rate fluctuations between groups had decreased; 44.7% of the experimental groups were either compacted 8-cell embryos or older, while 48.3% of the control group fell into this category. Finally, at the 75-79 hpc observation interval, the presence of lethal mutants within the experimental population became evident with 43.4% morulae in the experimental group versus 10.8% morulae in the control population.

Data presented in Table 1 show 36.3% presumed t^{w32}/t^{w32} lethal mutants. Interestingly, of 194 embryos from 18 control crosses which were recovered as cleavage stage embryos and cultured for over 35 hours, only one or 0.5% of this control population failed to undergo cleavage, compaction, morula formation, and cavitation. In another set of experiments using tailless Brachyury stock as parents (♂T/t^{w32} x ♀ T/t^{w32}) and ICR mice as controls (♂ICR x ♀ICR), 40.1% of the embryos in experimental crosses were presumed to be t^{w32} homozygotes (Table 2). Again, only a very low percentage (1.4%) of control embryos failed to develop to blastocyst stages.

TABLE 1

Development in vitro of embryos from ♂+/t^{w32} x ♀+/t^{w32} in comparison with non-lethal control (♂+/t^{w32} x ♀+/T) matings.

Mating	No. of females	Total no. of embs.	Avg. no. embs. per female	Dev't. to blasto-cyst	Dev't. blocked at morula	
Experimentals +/t^{w32} x +/t^{w32}	24	223	9.3[1]	141(63.2%)	82(36.8%)	36.3% presumed t^{w32}/t^{w32} lethal mutants
Controls +/T x +/t^{w32}	18	194	10.8	193(99.5)	1(0.5%)	

[1]In one experiment (Apr 7612), four females were flushed 24 hours late at 84 hpc versus 60 hpc, and only 14 embryos were recovered from the four females. By discounting those four females and their 14 embryos, the average number of embryos per female becomes 209/20 or 10.4.

TABLE 2

Development in vitro of embryos from T/t^{w32} x T/t^{w32} matings in comparison with non-lethal controls from ICR x ICR crosses.

Mating	No. of females	Total no. of embs.	Avg. no. embs. per female	Dev't. to blasto-cyst	Dev't. blocked at morula	
Experimentals T/t^{w32} x T/t^{w32}	7	53	7.6	31(58.5%)	22(41.5%)	40.1% presumed t^{w32}/t^{w32} lethal mutants
Controls ICR x ICR	7	74	10.6	73(98.6%)	1(1.4%)	

Our observations on experimental preimplantation mouse development afforded the following information. Some experimental embryos (presumed t^{w32}/t^{w32} mutants): (1) failed to undergo the normal 8-cell embryo compaction response, (2) became uncompacted as 16-cell embryos (early morulae), and (3) produced small blastocoelic spaces but failed to develop confluent blastocoels.

DISCUSSION

Present results on the generation of t^{w32} homozygous lethalities *in vitro* are consistent with previous findings. The 36.3% and 40.1% presumed t^{w32}/t^{w32} lethal mutant figures of Tables 1 and 2 respectively, agree well with the classical findings of Smith (1956), Mintz (1964), and others with regard to the t^{12} homozygote and with the more contemporary findings of Bennett and Dunn (1964), Hillman (1975), and Hillman and Hillman (1975) with respect to t^{w32} homozygotes. If as Hillman and Hillman (1975) suggest, t^{12} and t^{w32} are in fact separate alleles, then this study together with those of Hillman (1975), Hillman and Hillman (1975), and Bennett and Dunn (1964) represent the only attempts to characterize t^{w32} homozygotes *in vitro* as they undergo developmental arrest.

Observations on the developmental success of embryos from ♂ T/t^{w32} x ♀ T/t^{w32} matings indicate that lethal T/T embryos do not undergo arrest until after 96 hpc. Researchers who have investigated t^{12} and t^{w32} homozygotes have not used tailless mice (T/t^{12} or T/t^{w32}) as parents to produce lethal homozygotes, because ". . . the appearance of the t^{12}/t^{12} (or t^{w32}/t^{w32}) homozygote and the time of its death were unknown," and the ". . . offspring would have included the T/T homozygote and, possibly, abnormals of unknown causes arising as a result of inbreeding homozygosity" (Smith, 1956). Regarding inbreeding homozygosity, by conducting a sufficient number of matings and analyses, one may be able to iron out the effects due to depression. Secondly, T/T embryos which do not undergo abnormal development *in utero* until day eight (Chesley, 1935 and Bennett and Dunn, 1964) probably do not contribute significantly to the number of

arrested morulae in ♂ T/t^{w32} x ♀ T/t^{w32} matings. Thus abnormalities due to inbreeding homozygosity and precocious expression of the T/T lethality probably do not contribute significantly to the total number of arrested

morulae produced from T/t^{w32} parents. There may be advantages to the *in vitro* analysis of t^{w32} homozygotes derived from T/t^{w32} parents. Mutants derived from T/t^{w32} parents possess a different genetic background than those derived from $+/t^{w32}$ parents (the BALB/c background). Accordingly one might be able to detect phenomena of t^{w32} allele expression more readily in this different genetic background.

Cultural observations on lethal t^{w32} homozygotes from ♂ $+/t^{w32}$ x ♀ $+/t^{w32}$ matings reveal the following important points: (1) mutants may never completely compact, (2) some presumed mutants produce blastocoelic spaces but fail to form well defined blastocoels, and (3) in our experimental system the phenocritical period appears to extend over the 66-79 hpc interval. By correlating the presence of excess cytoplasmic lipids (a symptom of the t^{w32} homozygote) with failure to undergo normal 8-cell embryo compaction, Johnson and Granholm (1977) provide additional evidence that abnormal compaction is in fact a symptom of t^{w32} homozygotes. These data are consistent with findings of Hillman (1975) who reported that the t^{w32} homozygous phenocritical period begins with the 8-cell stage.

Correlation of t^{w32} expression with abnormal 8-cell embryo compaction would indeed be interesting. In addition to investigating developmental genetics of the T-locus, the investigation of t^{w32} homozygotes might also yield productive information on the compaction response. Continued *in vitro* observations should provide more specific information on the precise timing of the lethal t^{w32} allele and on morphological aspects of t^{w32} allele expression.

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