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Cytological analyses of factors which determine the number of primordial germ cells (PGCs) in *Xenopus laevis*

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SUMMARY

Correlation of the number of primordial germ cells (PGCs) at stage 47 with the amount of germ plasm at the 8-cell stage and with the number of the germ-plasm-containing cells (GPCCs) was analysed using two different laboratory-raised colonies of *Xenopus laevis*, HD and J groups. The average number of PGCs in J group tadpoles was significantly larger than that in HD group tadpoles. The amount of germ plasm in J group embryos was also demonstrated to be larger than in HD group embryos. The amount of germ plasm was related positively to the number of GPCCs at the 8-cell stage and to the resulting number of PGCs; embryos which contained larger amounts of germ plasm developed larger numbers of PGCs at stage 47.

The average number of PGCs in experimentally induced triploid tadpoles was exactly two-thirds of that in normal diploid tadpoles. Furthermore, in somatic cells (e.g. epidermis, muscle, pancreas), the number of cells in the triploid was also two-thirds of that in diploid tadpoles.

These findings suggest that the number of PGCs is regulated by at least two different mechanisms: first, the number of PGCs is primarily specified by the intrinsic amount of germ plasm in the fertilized egg. Second, it is regulated by an unknown mechanism which controls the total number of cells of whole embryos, such as the nucleocytoplasmic ratio.

INTRODUCTION

Germ plasm in anuran amphibian eggs is one of the best known examples of the cytoplasmic localization of a 'germinal determinant' (Smith, 1966; Buehr & Blackler, 1970), and another is the pole plasm in *Drosophila* eggs (see reviews by Beams & Kessels, 1974; Eddy, 1975; Smith & Williams, 1979). Morphological studies have shown that germ plasm exists even in unfertilized eggs as a number of small cytoplasmic islands (Czołowska, 1969). The germ plasm apparently is formed during oogenesis under the control of the maternal genome. Following fertilization, the germ plasm is believed to be partitioned equally into the four blastomeres during the first two cleavages, but segregated into only one daughter cell of each pair after the third cleavage (Whitington & Dixon, 1975). The partitioning or segregation of germ plasm during early cleavage, intracellular localization of the germ plasm during gastrulation and neurulation, morphological changes of the germinal granules, and migration of the germ-plasm-containing cells (GPCCs) (or presumptive PGCs) were extensively studied by many authors

Key words: PGCs, germ plasm, *Xenopus laevis*, triploid.

(see reviews by Nieuwkoop & Sutasurya, 1979; Dixon, 1981). According to Gipouloux (1975), germ cell determination occurs when the germ plasm occupies a juxtanuclear position in contact with the nuclear membrane. At present, however, it is still unknown how and when the germ plasm exerts its primary function.

Recently, Dixon (1981) proposed a hypothesis to explain the partitioning and/or segregation of the germ plasm and the establishment of the PGCs, by means of a 'founder clone' model. Basically, this model suggests the following events in order: (1) partitioning of the germ plasm, (2) its segregation, by means of asymmetrical mitoses, to an initial clone of presumptive PGCs, and (3) the proliferation of these founder clone cells by symmetrical mitoses in which germ plasm is distributed to both daughter cells.

The number of PGCs in the genital ridges of *Xenopus laevis* tadpoles reported from different laboratories is known to show a wide variation (e.g. Table 1 in Dixon, 1981). Preliminary observations in our laboratory indicated that two different laboratory-raised colonies of *Xenopus* showed different numbers of PGCs; tadpoles from one colony have a larger number of PGCs than the other. The factors which regulate the final number of PGCs is no doubt very complicated; e.g. intrinsic amount of germ plasm, number of cells in the founder clone (or GPCCs at early cleavage stages), rate of proliferation of founder clones, rate of successful migration of PGCs to the genital ridges, and so on. To know which factor(s) are the most important in regulating the number of PGCs we employed two experimental approaches. The first, the relationship between the volume of germ plasm, number of GPCCs, and resulting number of PGCs was intensively analysed in normal diploid embryos from two different colonies of *Xenopus laevis*. The second approach modified the relative amount of germ plasm to the nucleus of developing blastomeres (triploid embryos). Since the cleavage pattern of triploid eggs is identical to the normal diploid (Briggs, 1947), the nuclear–cytoplasmic ratio in triploid embryos is 1·5 times that in the diploids. If the germ plasm exerts its role by influencing the nucleus to specify PGCs, in triploid tadpoles in which the ratio of germ plasm to nucleus is two-thirds of the diploid, PGC differentiation will be altered. Accordingly, the number of PGCs in the triploid was examined and compared to that in the diploid tadpoles from corresponding spawnings.

MATERIALS AND METHODS

Source of embryos

The two colonies of *Xenopus laevis* which are maintained in our laboratory are referred to as J (formerly called G) and HD groups. The J group was established by extensive inbreedings and became histocompatible (Katagiri, 1978). The HD group is not inbred and is, therefore, wild type. Serial numbers were given to individual females so that precise comparisons could be made between individual spawnings. Eggs from hormone-stimulated females were rinsed briefly with 100 % Steinberg's solution and then inseminated with a sperm homogenate. Embryos were maintained at 23 °C. Developmental stages of the embryos and tadpoles were determined according to Nieuwkoop & Faber (1956). Stage-4 (8-cell) and stage-9 (late-blastula) embryos were treated with Helly's fixative for histological examination of the germ plasm. Stage-47 tadpoles were chosen for PGC counting.

PGC counts

To find the most convenient and reliable procedure for counting PGCs in stage-47 tadpoles three different procedures were tested: (1) direct observation with a dissecting microscope of the PGCs which had been dissociated from the genital ridges of unfixed tadpoles (Wylie & Roos, 1976; Subtelny, 1980); (2) direct observation of the genital ridges of fixed material with a scanning electron microscope (Wylie, Bancroft & Heasman, 1976); and (3) histological examination of serial sections through the genital ridges (Ijiri & Egami, 1975; Wakahara, 1977). The first method was the simplest. However, the data were unreliable; it was very difficult to distinguish PGCs from somatic cells by this procedure. The second method (scanning electron microscope) was much easier than histological examination, but inevitably underestimated PGC numbers. Two or three PGCs were frequently grouped together to make one lump along the genital ridges making it difficult to estimate accurately the number of PGCs from surface structure. Thus, histological examination was employed to determine accurately the PGC numbers, although it took a lot of time to prepare histological sections and to examine them with a light microscope. PGC numbers were carefully determined by counting the nuclei of PGCs through serial sections from the cloacal position rostrally until the genital ridges disappeared.

Somatic cell counts

The number of somatic cells which constitute the tail-fin epidermis, myotome and developing pancreas at stage 47 was determined as follows: the nuclei in these tissues in the unit area of histological sections were counted under a light microscope, and the square root of that value multiplied twice by itself, in the case of myotome and pancreas.

Measurement of germ plasm volume

The volume of the germ plasm in 8-cell embryos was determined as follows. Serial horizontal sections of embryos were stained with Heidenhain's Azan. Every section from each embryo that contained the germ plasm was taken, and the outline of each patch of germ plasm was traced onto section paper using a camera lucida. By multiplying the thickness of the histological sections (7 μm) by the total area of the germ plasm the volume of the germ plasm was estimated. The number of the GPCCs in 8-cell and late-blastula embryos was also counted.

Production of triploid embryos

Triploid embryos were obtained by suppressing second polar body emission with a brief cold treatment (2°C) of the normally fertilized eggs (Kawahara, 1978). A small piece of the tail-fin epidermis of stage-47 tadpoles was removed and examined for number of the nucleoli per nucleus. Since each genome in this species has one nucleolar organizer, the number of the nucleoli was a good index of ploidy.

RESULTS

Observations of normal diploid embryos from two different colonies

PGC number

Table 1 shows individual PGC counts at stage 47 in the two colonies (HD and J groups). One hundred and eleven tadpoles from 10 different spawnings in the HD group and 128 tadpoles from 10 different spawnings in the J group were examined. The number of PGCs per tadpole in the HD group showed a wide variation from tadpole to tadpole (e.g. from 0 to 45 in HD 3), and spawning to spawning (e.g.

from 6·5 in HD 50 to 42·9 in HD 1). In the J group, however, the variation in the number of PGCs was much smaller than in the HD group, especially from spawning to spawning (e.g. from 37·9 in J 37 to 57·5 in J 36). Furthermore, the average numbers of PGCs from 10 spawnings in the J group was much larger than in the HD group. To test whether the difference in the average number of the PGCs in the two colonies was significant or not, a statistical analysis was done. On the basis of the Student's t-test ($P = 0·05$), the difference of the average numbers of the PGCs between HD and J groups is significant as follows:

$$t\text{cal} = 3·64 > t0·05 = 2·101 \ (v = 18).$$

Therefore, it was demonstrated that the number of the PGCs in stage-47 tadpoles from the J group was significantly larger than in the HD group.

Germ plasm amount

At stage 4 (8-cell stage), the germ plasm was easily detected histologically as large cytoplasmic patches which were distinctive compared to the surrounding yolky cytoplasm. Table 2 contains data on the volume of germ plasm in individual embryos from the two groups. 45 embryos from six different spawnings in the HD

Table 1. *Number of PGCs at stage 47 in two colonies (HD and J) of* Xenopus laevis

Serial no. of female	No. of tadpoles examined	No. of PGCs per tadpole	Average no. of PGCs ± s.e.
HD 1	10	1, 17, 33, 44, 52, 53, 54, 54, 57, 60	42·9 ± 6·1
HD 3	10	0, 7, 11, 12, 18, 19, 21, 25, 32, 45	19·0 ± 4·1
HD 5	17	10, 15, 17, 18, 18, 22, 22, 25, 25, 26, 26, 27, 28, 28, 29, 33, 34	23·7 ± 1·6
HD 50	10	0, 0, 0, 0, 0, 1, 8, 11, 16, 29	6·5 ± 3·1
HD 52	9	16, 23, 25, 31, 31, 32, 40, 41, 43	33·1 ± 3·0
HD 57	12	1, 2, 12, 12, 17, 18, 20, 22, 26, 30, 30, 33	18·6 ± 3·0
HD 58	8	9, 12, 18, 23, 26, 27, 28, 43	23·3 ± 3·7
HD 65	10	0, 7, 9, 12, 14, 19, 23, 33, 35, 37	18·4 ± 4·0
HD 68	12	4, 9, 9, 10, 11, 12, 13, 17, 19, 22, 22, 22	14·2 ± 1·8
HD 71	13	6, 7, 7, 7, 7, 13, 15, 18, 22, 22, 22, 25, 26	15·2 ± 2·1
(total)	(111)		(21·5 ± 2·9)
J 36	11	39, 41, 46, 46, 56, 57, 59, 65, 66, 72, 85	57·5 ± 4·3
J 37	16	23, 24, 25, 28, 29, 32, 35, 36, 38, 39, 39, 41, 45, 50, 54, 68	37·9 ± 3·0
J 42	10	24, 27, 36, 37, 44, 47, 53, 54, 55, 62	43·9 ± 4·0
J 47	12	30, 32, 38, 53, 57, 58, 60, 62, 62, 66, 71, 86	56·3 ± 4·7
J 52	17	8, 28, 28, 29, 35, 36, 39, 41, 41, 46, 48, 59, 61, 64, 65, 68, 77	45·5 ± 4·4
J 54	9	19, 25, 42, 43, 45, 45, 49, 49, 52	41·0 ± 3·8
J 55	11	37, 40, 42, 45, 46, 48, 49, 59, 72, 89, 90	56·1 ± 5·8
J 68	12	13, 17, 17, 19, 30, 50, 56, 56, 58, 64, 64, 72	43·0 ± 6·4
J 73A	7	16, 26, 32, 34, 49, 51, 65	39·0 ± 6·3
J 73B*	13	13, 18, 35, 43, 43, 44, 49, 50, 50, 51, 69, 72	44·6 ± 3·6
(total)	(128)		(46·5 ± 4·6)

* The second spawning was induced about 2 months after the first one with the same female, J 73.

group and 43 embryos from six different spawnings in the J group were examined. As was the case for PGC number the volume of germ plasm per embryo in the HD group showed a wide variation from embryo to embryo (e.g. from $53 \times 10^2 \, \mu m^3$ to $948 \times 10^2 \, \mu m^3$ in HD 65) and from spawning to spawning (e.g. from $179 \cdot 8 \times 10^2 \, \mu m^3$ in HD 71 to $1066 \times 10^2 \, \mu m^3$ in HD 1). In the J group, however, the variation in the volume of germ plasm was much smaller than in the HD group (e.g. from $834 \cdot 1 \times 10^2 \, \mu m^2$ in J 37 to $1241 \cdot 7 \times 10^2 \, \mu m^3$ in J 55). Furthermore, the embryos from the J group appeared to have much more germ plasm than the embryos from the HD group. To test whether the difference was significant, a statistical calculation was done. On the basis of Student's t-test ($P = 0 \cdot 1$), the difference between the average volume of the germ plasm in the two groups was significant. Thus, the embryos in the J group were demonstrated to have a larger amount of germ plasm than those in the HD group (about twofold).

GPCC number

To understand the possible relationship between GPCC number at the 8-cell and late-blastula stages and the resulting number of PGCs at stage 47, GPCC number was counted in 70 embryos from six spawnings in the HD group and 69 embryos from six spawnings in the J group. Table 3 shows the average numbers of the GPCCs in 8-cell and late-blastula embryos. In spite of the significant difference in the number of PGCs in stage-47 tadpoles between the HD and J groups, there were no fundamental differences in GPCC numbers in 8-cell or late-blastula embryos between the two groups.

Table 2. *Total volume of germ plasm per embryo at the 8-cell stage in two colonies of* Xenopus laevis

Serial no. of female	No. of embryos examined	Volume of germ plasm per embryo ($\mu m^3 \times 10^2$)	Average vol. of germ plasm ± S.E. ($\mu m^3 \times 10^2$)
HD 1	5	922, 975, 1088, 1154, 1194	1066·6 ± 51·8
HD 3	7	310, 407, 518, 628, 676, 715, 787	577·3 ± 65·3
HD 5	8	696, 735, 759, 764, 766, 874, 904, 909	800·9 ± 29·1
HD 65	10	53, 82, 146, 204, 304, 350, 405, 453, 491, 948	343·6 ± 82·7
HD 68	6	389, 397*, 408, 410, 411, 525	423·3 ± 20·6
HD 71	9	29, 88, 95, 171, 182, 223, 269, 271, 290	179·8 ± 30·9
(total)	(45)		(565·2 ± 46·7)
J 36	7	1031, 1139, 1228, 1248, 1265, 1354, 1380*	1235·0 ± 45·5
J 37	10	664, 781, 787, 809, 812, 858, 880, 881, 931*, 938	834·1 ± 25·9
J 42	7	894, 912, 930, 930, 982, 1079, 1276	1000·4 ± 51·5
J 54	6	641, 759, 770, 862, 912, 1119	843·8 ± 67·0
J 55	6	927, 1008, 1176, 1284, 1341, 1714	1241·7 ± 114·4
J 73	7	944, 946, 1042, 1152, 1244, 1281, 1310	1131·3 ± 58·7
(total)	(43)		(1046·5 ± 60·5)

*In these embryos, the partitioning of the germ plasm into 4 blastomeres was analysed in Table 4.

Germ plasm partitioning

The amount of germ plasm in individual blastomeres was measured to analyse the way in which germ plasm is partitioned during the first two cleavages. Table 4 contains typical data of the volume of the germ plasm in the four vegetal blastomeres in three eggs chosen from Table 2. The germ plasm was rather unevenly partitioned into the four blastomeres. In one extreme case, one blastomere contained approximately 150 times the volume of germ plasm in the other blastomere.

Correlation of PGC number with germ plasm amount

From the results described above it is assumed that an embryo which contains more germ plasm at the 8-cell stage develops more PGCs at stage 47, and *vice versa*. In order to test that assumption, statistical analyses were performed. Fig. 1 shows the correlations of the number of PGCs with (A) germ plasm volume; (B) GPCC number at 8-cell stage; and (C) GPCC number at the late-blastula stage.

Table 3. *GPCC number in 8-cell and late-blastula embryos in the two colonies of* Xenopus laevis

	8-cell embryo		late-blastula embryo	
Serial no. of female	No. of embryos examined	Average no. of GPCCs per embryo ± s.e.	No. of embryos examined	Average no. of GPCCs per embryo ± s.e.
HD 1	5	3.8 ± 0.2	11	4.8 ± 0.5
HD 3	7	3.6 ± 0.3	7	5.9 ± 0.6
HD 5	8	4.0 ± 0.0	13	4.9 ± 0.4
HD 65	10	2.9 ± 0.3		
HD 68	6	3.3 ± 0.3		
HD 71	9	2.7 ± 0.3		
(total)	(45)	(3.4 ± 0.2)	(31)	(5.2 ± 0.5)
J 36	7	4.0 ± 0.0	9	5.7 ± 0.6
J 37	10	3.5 ± 0.3		
J 42	7	3.3 ± 0.2	6	4.8 ± 0.3
J 54	6	3.8 ± 0.2		
J 55	6	3.8 ± 0.2	11	7.5 ± 0.6
J 73	7	3.4 ± 0.4		
(total)	(43)	(3.6 ± 0.2)	(26)	(6.0 ± 0.5)

Table 4. *Partitioning of the germ plasm into 4 vegetal blastomeres in 8-cell stage embryos*

Serial no. of female	No. of embryos examined	Volume of germ plasm per blastomere ($\mu m^3 \times 10^2$)	Total volume of germ plasm per embryo ($\mu m^3 \times 10^2$)
HD 68	1	2, 37, 51, 307	397
J 36	1	20, 152, 505, 703	1380
J 37	1	83, 161, 162, 525	931

Among these three, only the correlation of the resulting number of PGCs with germ plasm volume was confirmed to be significant on the basis of Student's t-test $(P = 0.05)$. Correlations of number of PGCs at stage 47 with the numbers of GPCCs at the 8-cell (Fig. 1B) and late-blastula (Fig. 1C) stages were not significant.

Correlation of GPCC number with germ plasm amount

Fig. 2 shows the relationship between amount of germ plasm and number of GPCCs at the 8-cell (A) and late-blastula (B) stages. It was clear that the volume of germ plasm was positively correlated with the number of GPCCs at 8-cell but not at late-blastula stages. On the basis of Student's t-test $(P = 0.05)$, the correlation of the amount of germ plasm with the number of GPCCs at the 8-cell stage was significant.

Observations on triploid tadpoles

Characteristics of triploid

The results described in Table 2 indicate that germ plasm volume was relatively constant within one spawning in the J group, whereas some variation was observed
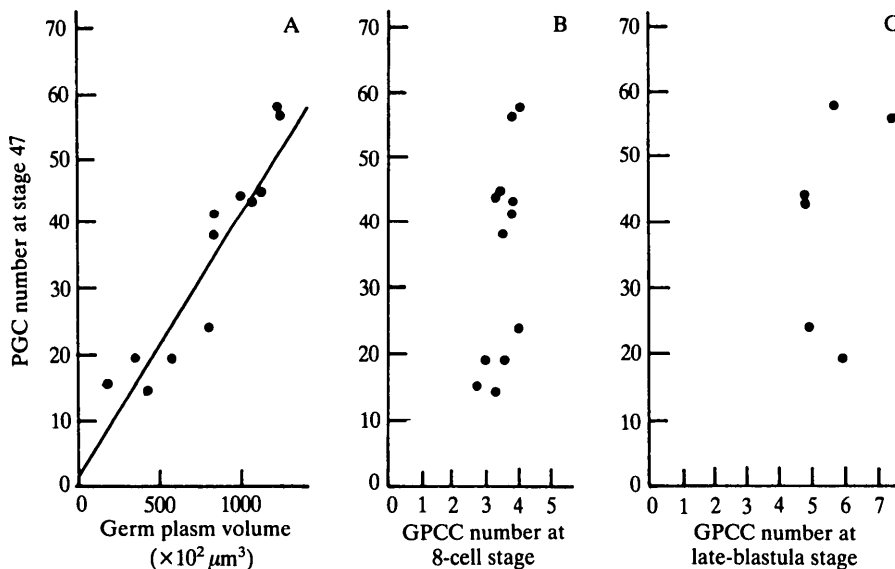

Fig. 1. Correlations of PGC number in stage-47 tadpoles with (A) germ plasm amount in 8-cell embryos, (B) GPCC number at 8-cell stage, and (C) GPCC number at the late-blastula stage. Average PGC numbers from 12 spawnings (A and B) or 6 spawnings (C) in HD and J groups are plotted against the average volume of germ plasm per embryo (A), average GPCC number at 8-cell (B) and late-blastula (C) stages from the corresponding spawnings. On the basis of Student's t-test $(P = 0.05)$, only the correlation of the number of PGCs with the amount of germ plasm is statistically positive.

in eggs from one spawning in the HD group. Therefore, J group animals were employed exclusively in further experiments.

Fig. 3 shows the morphological features of tail-fin epidermis from normal diploid and experimentally induced triploid tadpoles. It was relatively easy to distinguish triploid from diploid cells because triploid cells were usually larger and contained three nucleoli per nucleus (Fig. 3B), whereas the diploid cells were smaller and contained one or two nucleoli (Fig. 3A). Triploid embryos grew and differentiated at the same rate as diploids despite the fact that the triploid cells were apparently larger than diploid cells.

PGC number

Morphological characteristics of PGCs in triploid tadpoles were identical to those in the diploid controls (Fig. 3C,D). Table 5 shows the average number of PGCs in diploid and triploid tadpoles at stage 47. 56 triploid tadpoles from five different spawnings and 67 diploid tadpoles from corresponding spawnings were examined. In every spawning the number of PGCs in triploid tadpoles was exactly two-thirds that in diploid tadpoles. For example, the average number of PGCs in
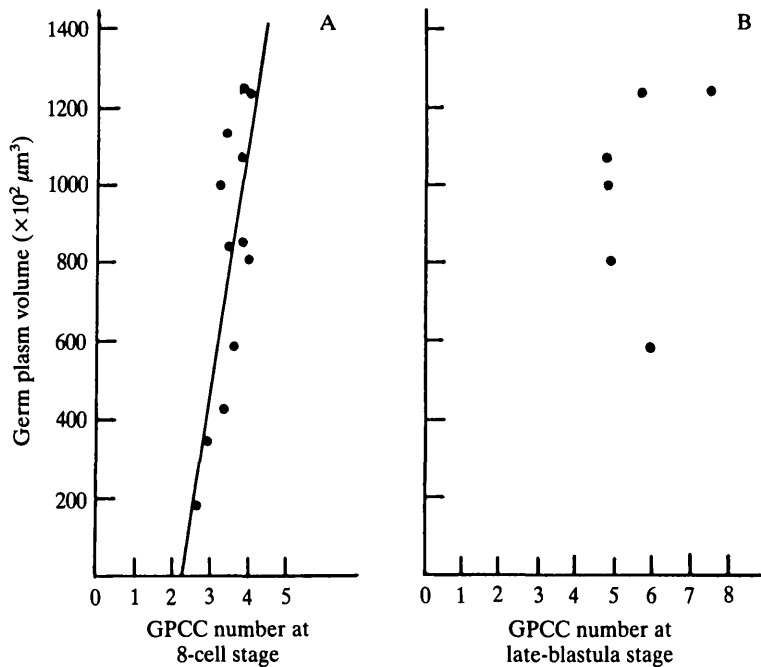

Fig. 2. Correlations of germ plasm amounts in 8-cell embryos with (A) GPCC number at the 8-cell stage, and (B) GPCC number at the late-blastula stages. Average volumes of germ plasm from 12 spawnings (A) or from 6 spawnings (B) in HD and J groups are plotted against average GPCC number at 8-cell (A) and late-blastula (B) stages from the corresponding spawnings. On the basis of Student's t-test ($P = 0.05$), the correlation of the volume of the germ plasm with the number of the GPCCs at 8-cell stage is statistically positive.

triploid tadpoles from J52 was 30·0, while the diploid tadpoles from the same spawning had an average of 45·5 PGCs.

Somatic cell numbers

The numbers of somatic cells in triploid tadpoles at stage 47 were compared to those in diploid tadpoles from corresponding spawnings. In the tail-fin epidermis
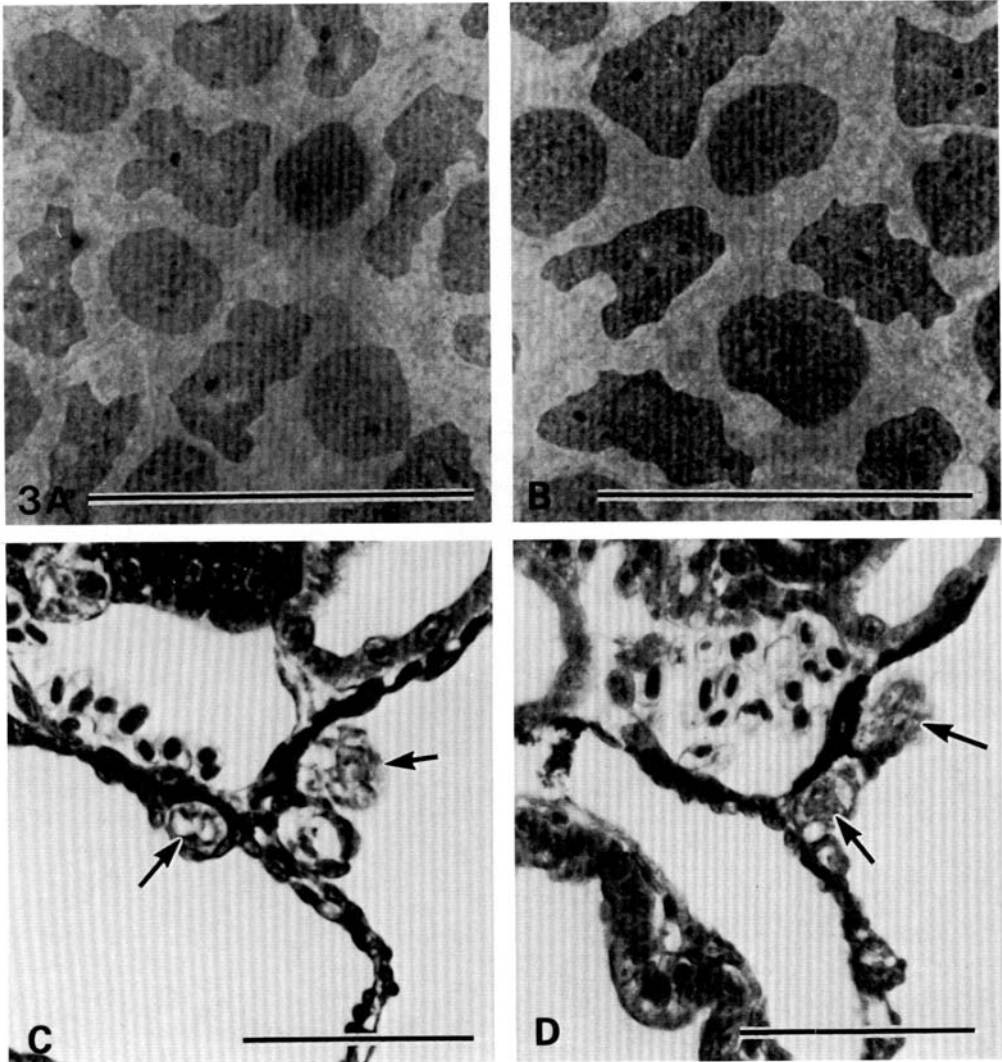

Fig. 3. Photomicrographs of cells from diploid and triploid stage-47 tadpoles. (A) Diploid cells from tail-fin epidermis showing nuclei with two nucleoli per nucleus. (B) Triploid cells from tail-fin epidermis showing nuclei with three nucleoli per nucleus. The triploid cells are somewhat larger than the diploid cells. (C) The appearance of PGCs (arrows) in the genital ridges of a diploid tadpole. (D) The appearance of PGCs (arrows) in the genital ridges of a triploid tadpole. No significant difference in the morphological properties of PGCs between diploid and triploid tadpoles were detected. Delafield's haematoxylin–eosin stained. Bar indicates $50\,\mu$m.
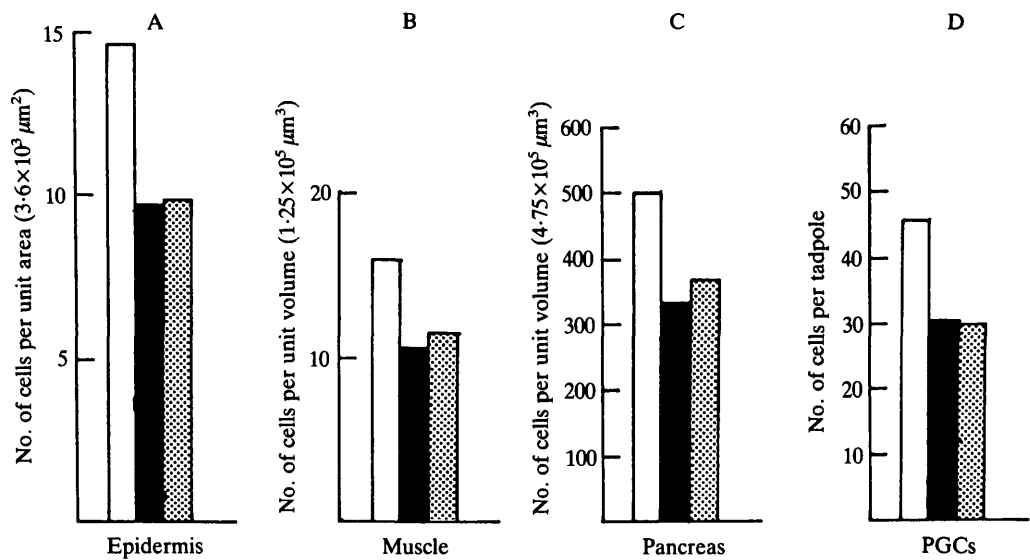
Fig. 4. Comparisons of numbers of somatic cells and PGCs at stage 47 between diploid (open columns) and triploid (dotted columns) tadpoles. Closed columns indicate the two-thirds levels of numbers of cells in diploid controls for references. The number of epidermal cells (A) is expresssed as the one in unit area. The numbers of the muscle (B) and pancreas (C) cells are expressed as the ones in unit volume. The number of PGCs is expressed as number of cells per tadpole. In all cell types examined the number of cells from triploid tadpoles are approximately two-thirds of those from diploid controls.

the cell number per unit area was compared because this cell type could be considered to be approximately two dimensional. In developing muscle cells and pancreatic cells, however, the number of cells per unit volume was compared because these were three dimensional in nature. Fig. 4 illustrates the comparisons between the numbers of somatic cells in triploid and diploid tadpoles. In every cell type the number of cells in triploid tadpoles was approximately two-thirds that in diploid ones. The size of individual cells of triploids was much larger than that of

Table 5. *Average PGC number at stage 47 in diploid and triploid tadpoles of* Xenopus laevis

	Diploid		Triploid	
Serial no. of female	No. of tadpoles examined	Average no. of PGCs per tadpole ± s.e.	No. of tadpoles examined	Average no. of PGCs per tadpole ± s.e.
J 37	16	37.9 ± 3.0	17	22.5 ± 3.2
J 47	12	56.3 ± 4.7	6	34.3 ± 6.1
J 52	17	45.1 ± 4.4	14	30.0 ± 2.9
J 54	9	41.0 ± 3.8	6	30.5 ± 7.1
J 73	13	44.6 ± 4.6	14	35.6 ± 3.5
(total)	(67)	(45.1 ± 4.1)	(57)	(30.6 ± 4.5)

diploids. Since the size of triploid embryos was nearly identical to diploids from the egg to stage-47 tadpole, the total number of cells which constituted the whole triploid tadpole appeared to be two-thirds that of diploid tadpoles.

DISCUSSION

This is the first report to show there are genetic differences among *Xenopus laevis* with regard to the number of PGCs in stage-47 tadpoles. When over 200 tadpoles from ten different spawnings in each of two colonies were examined, the difference in the average number of PGCs between the two colonies (HD and J groups) was demonstrated to be statistically significant.

Less variation in the number of PGCs in different spawnings from the J group is consistent with the fact that the J group is a genetically much more pure, histocompatible colony (Katagiri, 1978). The HD group, which is a genetically wild-type colony, shows a wide variation in the number of PGCs from tadpole to tadpole and from spawning to spawning (Table 1). The range of the variation in the number of PGCs in *Xenopus* tadpoles which has been reported from different laboratories (Table 1 in Dixon, 1981) is very similar to the range of the variation in the number of PGCs in the HD group reported here. This suggests that a wide variation in the number of PGCs will inevitably be encountered if wild-type *Xenopus* are used. We, therefore, recommend that a genetically pure *Xenopus*, such as the J group, should be employed as experimental animals in order to obtain consistent PGC counts.

Of the factors which determine the final number of PGCs, the intrinsic amount of germ plasm at the 8-cell stage was demonstrated to correlate positively with the resulting number of PGCs at stage 47. That is, an embryo which has more germ plasm at the 8-cell stage develops more PGCs at stage 47. This is consistent with the results of earlier experiments which showed that a withdrawal of germ plasm from fertilized eggs resulted in a decrease in the final number of PGCs (Buehr & Blackler, 1970). Conversely, microinjection of a germ plasm fraction into eggs results in an increase in the number of PGCs (Wakahara, 1978). Those observations further support the idea that the germ plasm contains the 'germinal determinant' (Ikenishi, Okuda & Nakazato, 1984).

If every GPCC in late blastulae proliferates by symmetrical divisions to form PGCs (Dixon, 1981), the number of GPCCs in the late-blastula stage should correlate directly with the resulting number of PGCs. However, the results obtained in this study demonstrated that the number of GPCCs in late blastulae did not correlate with the final number of PGCs at stage 47 (Fig. 1C). These rather curious results can be explained as follows (Fig. 5): because the germ plasm is not partitioned equally into the four vegetal blastomeres during the first two cleavages the amount of germ plasm in individual vegetal blastomeres of 8-cell embryos varies considerably (Table 4). Since from the 8-cell to blastula stages the germ plasm is segregated into one daughter cell of each pair by means of asymmetrical divisions (Dixon, 1981), the amount of germ plasm must vary in individual

Expected partitioning and/or segregation
during three cell divisions

Expected no. of PGCs
in a typical case
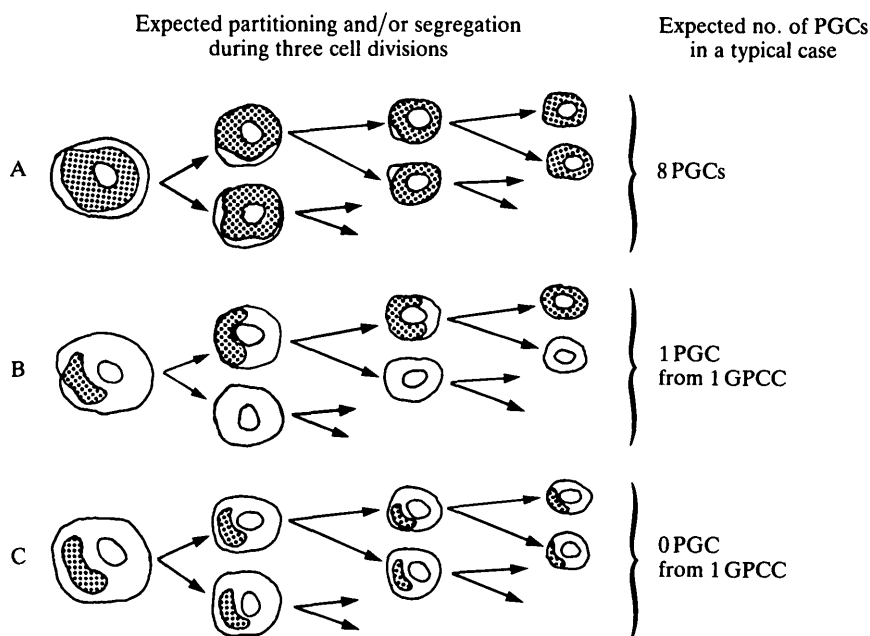
A

8 PGCs

B

1 PGC
from 1 GPCC

C

0 PGC
from 1 GPCC

Fig. 5. Schematic illustration of partitioning and/or segregation of the germ plasm and establishment of the PGCs after the late-blastula stage for hypothetical explanations of the regulatory mechanisms of PGC number. Dotted area indicates the presence of germ plasm. (A) When one GPCC receives a sufficient amount of germ plasm by late-blastula stage, the germ plasm will be partitioned into both daughter cells in each pair during the subsequent three divisions, resulting in eight presumptive PGCs from one GPCC, as in the 'founder clone' model of Dixon (1981). If one GPCC receives an insufficient amount of germ plasm, two different explanations are possible; (B) 'segregation model' or (C) 'dilution model'. In the segregation model, the germ plasm is segregated into only one daughter cell of each pair until the cell acquires the sufficient amount of germ plasm to surround the nucleus. In a typical case, only one presumptive PGC is formed from one GPCC. In the dilution model, the germ plasm is partitioned into both daughter cells of each pair during subsequent cell divisions, resulting in 'dilution' of the germ plasm in the descendent cells. In an extreme case in this model, no presumptive PGCs are established from a GPCC. Wide variation in the number of the PGCs in stage-47 tadpoles of *Xenopus laevis* can be explained by a combination of these models.

GPCCs. When a GPCC receives enough germ plasm to surround the nucleus entirely at the late-blastula stage (Blackler, 1958; Whitington & Dixon, 1975), it can be expected that the germ plasm will be partitioned equally into both daughter cells of each pair during the subsequent three cell divisions, resulting in eight presumptive PGCs from one GPCC as postulated by Dixon's founder clone model (upper row in Fig. 5). On the other hand, in a GPCC which receives an insufficient amount of germ plasm, two possible mechanisms are proposed; the 'segregation model' and the 'dilution model'. In the segregation model (middle row in Fig. 5), the germ plasm must be segregated into only one daughter cell by asymmetrical divisions, just as in the preceding cleavages from 8-cell to late-blastula stage (Dixon, 1981). In an extreme case of this model, only one PGC is established from

one GPCC. In the dilution model (lower row in Fig. 5), the germ plasm is partitioned equally into both daughter cells by asymmetrical divisions during the subsequent cell divisions. As a result, the amount of germ plasm per cell becomes too small to surround the nucleus when occupying a juxtanuclear position (Whitington & Dixon, 1975). In this case, no PGCs are expected to develop from a GPCC because an insufficient amount of germ plasm leads either to failure of the establishment of the cell as a PGC (cf. Wakahara, Neff & Malacinski, 1984) or to failure in the protection of the nucleus from becoming channelled into a somatic pathway (Smith, 1965; Wakahara, 1982). These hypothetical explanations make it possible to understand the wide variation in the PGC number in wild-type *Xenopus*. These models also agree with two sets of data: (1) that PGC number is correlated directly with the amount of germ plasm per egg and with the number of GPCCs at the 8-cell stage, but not with the GPCC number at late-blastula stage; and (2) PGC numbers reported in this study (Table 1) are not a simple multiple of eight as expected in the founder clone model.

The fact that PGC number in triploid tadpoles is exactly two-thirds of that in diploid controls is consistent with the concept that the germ plasm exerts its role by influencing the nucleus to specify GPCCs to become PGCs (Ijiri & Egami, 1976) or by protecting the nucleus and thus preventing the cells from becoming somatic cells (Smith, 1965), because the relative amount of the germ plasm per nucleus in triploid embryonic cells is two-thirds that in diploid controls.

Another important conclusion concerning regulation of PGC number can be drawn from a comparison of the number of cells between triploid and diploid embryos. Not only the number of PGCs but also the number of other somatic cells in triploid tadpoles is two-thirds of those in diploid controls. General mechanisms for regulation in cell size and mitotic rate in triploid embryos have already been fully discussed by Fankhauser (1945) and Briggs (1947). They proposed that the nuclear–cytoplasmic ratio causes the regulation of mitotic rate to compensate for differences in cell size between diploid and triploid embryos. In respect to the nuclear–cytoplasmic ratio, Newport & Kirschner (1982*a,b*) have reported that new cellular activities at the blastula stage such as RNA transcription and motility of cells are regulated by a critical ratio of nucleus to cytoplasm. Taken together with results obtained in this study, it can be assumed that the number of PGCs as well as somatic cells is controlled by a general regulatory mechanism such as the nuclear–cytoplasmic ratio which governs the total number of cells in the whole embryo. Thus, the number of PGCs is regulated by at least two different mechanisms: first, the intrinsic amount of germ plasm in the fertilized eggs; second, a common but unknown mechanism which controls the total number of cells in whole embryos.

Since the number of GPCCs at the late-blastula stage in triploid embryos was identical to that in diploid controls (data not shown), the difference in germ cell number between triploids and diploids must appear after the late-blastula stage. This is consistent with earlier observations that there are no detectable differences in cell cycles and in cell size between triploid and diploid embryos during early

cleavage stages (Briggs, 1947), and with recent findings that onset of new cellular activities in blastomeres is acquired only after the mid-blastula stage (Newport & Kirschner, 1982a).

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