NUCLEAR TRANSFER STUDIES OF AMOEBA PROTEUS AFTER THE INHIBITION OF CELL DIVISION BY INJECTION OF NON-HOMOLOGOUS CYTOPLASM.

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SUMMARY

Following the injection of the post-microsomal supernatant fraction of Amoeba discoides cytoplasm into A.proteus, cell division is inhibited in at least 90% of the recipient cells. Nuclear transfers were performed to determine the site of inhibition in these injected cells. When nuclei from injected,inhibited cells one day after injection were transferred into new A.proteus cytoplasms, 62% of the transfers divided. This ability to promote division declined with the length of time between transfer and the original injection. However, when nuclei from A.proteus were transferred into injected, inhibited cytoplasms, only a low number of cells divided, comparable to the number obtained after the injection operation only, namely less than 10%. Thus although many nuclei could recover from inhibition, it was not possible to restore the cytoplasms of inhibited cells by new nuclei.

A feature of cytoplasmic injection between strains of large, free-living amoebae is an incompatibility phenomenon, where injection of non-homologous cytoplasm is followed by the inhibition of cell division in many of the recipient cells. When cytoplasm taken from Amoeba discoides was injected into A.proteus, some 60% of the injected cells failed to divide and remained single cells until death some two to three weeks later (1). A similar result has been reported after the injection of A.proteus cytoplasm into the large, multi-nucleate Chaos carolinensis (2).

Examination of fractions prepared from A.discoides cytoplasm showed that the highest level of inhibition of cell division, (80%), followed the injection of the post-microsomal supernatant fraction (3). Preparation of this fraction from larger numbers of A.discoides gives a more active fraction inhibiting cell in 90-95% of recipient A.proteus (4). Jeon and Lorch (5) injecting supernatant material from A.proteus into A.discoides obtained a similar level of inhibition

(93%), and attributed this failure of cell division to "lethal factors" (6) present in the cytoplasm of amoebae.

Little is known of the events which follow injection of non-homologous cytoplasm or of the nature of the division inhibitor. Injection of a variety of materials including DNA, RNA, proteins or homologous cytoplasm is followed by 90% division of the recipient cells (4). Some studies have indicated that the division inhibitor is protein of high molecular weight (5), but its identity and normal relationship to cellular events is unknown.

The technique of nuclear transfer provides a means of assessing the activity of inhibitory molecules on nucleus and cytoplasm, and has been used in studies of amoebae after growth in actinomycin D (7) and dimidium bromide (8). We have made nuclear transfers between injected and thus inhibited cells, and normal A.proteus, using "inhibited" nuclei and normal cytoplasms and vice versa at various times after the initial injection of non-homologous cytoplasm.

MATERIAL AND METHODS

Amoeba proteus ($_{T1}$P) and A.discoides ($_{T1}$D), originally obtained from Taylor (Glasgow) in 1948 and since then maintained in the Zoology Dept., King's College, were grown in "wheat grain" cultures (9) in Chalkley's medium. Large numbers of A.discoides used in the preparation of the post-microsomal supernatant fraction were grown using a method modified from Griffin (10) and fed on Tetrahymena pyriformis. Cells of A.discoides were homogenised in sucrose-TKM* buffer(3), and the post-microsomal supernatant fraction obtained after centrifugation of this homogenate for 2½ hr at 105,000g. The material was stored in small aliquots at -20°C until used. A small volume of this supernatant material was injected into A.proteus using techniques described previously (1). Each preparation of the supernatant fraction was tested for its efficiency in inhibiting cell division by injecting 100 cells of A.proteus and determining the number of cells dividing, usually from 5-10%.

* TKM is 50mM Tris-HCl, 25 mM KCl, 5mM $MgCl_2$, pH 7.4

Table 1.

Transfer of nuclei from A.proteus into the cytoplasm of injected, inhibited A.proteus.

Time after injection of A.discoides supernatant material**	No. of transfers	No. of cells dividing	% Division+
4 hr	68	0	0
1 day	105	8	7.6
2 days	91	7	7.7
4 days	50	3	6.0
6 days	44	1	2.4
9 days	22	1	4.5

** % division of control cells injected with post-microsomal fraction from A.discoides was 8%

+ Division of homotransfers (control nucleus into control cytoplasm) = 95%

Nuclear transfers were made between nuclei from normal A.proteus and cytoplasm of injected inhibited A.proteus and vice versa, using techniques described previously (11). Cells were used at known times after injection of supernatant material, from 4 hr to 14 days. After transfer, cells were kept singly in solid watchglasses containing 2 ml Chalkley's medium and food organisms (Colpidium sp., 500/ml) until they divided or died. Those cells that divided were maintained to see whether they would form clones.

RESULTS

When nuclei from normal A.proteus were transferred into enucleate cytoplasms of injected cells only a few transfers divided (Table 1). The low number of dividing cells was comparable to the low number of cells which failed to be inhibited following the injection of non-homologous cytoplasm, i.e. from 5-10%. Even if the nuclear transfer was performed a relatively short time after the

Table 2.

Transfer of nuclei from injected, inhibited A.proteus into the cytoplasm of A.proteus.

Time after injection of A.discoides supernatant material**	No. of transfers	No. of cells dividing	% Division+
4 hr	96	45	47
1 day	103	64	62
2 days	93	29	31
4 days	87	13	15
6 days	53	5	9.4
9 days	27	0	0
14 days	24	0	0

** % division of control cells injected with post-microsomal fraction from A.discoides was 8%.

+ Division of homotransfers (control nucleus into control cytoplasm)= 95%

initial injection, namely 4 hr, the injected cell cytoplasm failed to support division in the transfer.

The results obtained after the transfer of nuclei from injected cells into normal, enucleate cytoplasms showed that many of these nuclei could support division in normal cytoplasms (Table 2). The number of transfers that divided depended on the length of time between injection and transfer, the highest number dividing (62%)was obtained if the transfer was performed 1 day after injection. This percentage fell with time, reaching the level obtained in control cells injected with non-homologous cytoplasm if the transfer was performed 6 days after injection. The lower number of cells dividing after 4 hr (47%) was probably due to mechanical damage caused by two operations in a relatively short time. All the cells that divided, from both sets of transfers, successfully gave rise to clones. When cells from these clones were injected with non-

homologous cytoplasm, they were inhibited and failed to divide.

DISCUSSION

The results obtained show that a good proportion of nuclei from inhibited cells were able to divide when placed in normal cytoplasm. However, it was not possible to reverse the damage to the injected cell cytoplasm by the introduction of a normal nucleus. This failure could not be attributed to loss of viability of cytoplasm, since Ord (12) has shown that even after 6 days enucleate cytoplasms are 70% viable.

These results are similar to those obtained in earlier studies where division was inhibited using either actinomycin D (7) or dimidium bromide (8). In both instances a percentage of drug-treated nuclei were able to divide in new cytoplasm, but it was not possible to induce division in drug-treated cytoplasms by the introduction of new nuclei. The survival of the drug-treated nuclei, and the nuclei from "injection-inhibited" cells might be due to the release of bound material by incoming cytoplasmic components, and the amounts released into the cytoplasm in many instances were not inhibitory. The irreversible damage to the cytoplasm is less easily understood. When drugs were used, then mitochondrial DNA might have been impaired, since both compounds bind to DNA. A new nucleus would not be expected to reverse such cytoplasmic damage. The nature of the inhibiting material contained in the post-microsomal supernatant fraction injected into A.proteus has been suggested to be protein, and this fraction is rich in protein and RNA. Of the proteins present in these strains of amoebae, there is a class which migrate between cytoplasm and nucleus, called " rapidly migrating proteins" (13), and which migrate into a non-homologous nucleus (14). These are a heterogeneous group of proteins, but the only one studied is of low molecular weight (14). However, it might be that "injected inhibitor" bound at cytoplasmic sites in inhibited cells is also able to migrate into the new introduced nucleus in sufficient amounts as to inhibit activity. It is now known that nuclear transfer results in flow of material from

the nucleus, probably as a result of mechanical damage, and this material is gradually replaced from the cytoplasm (13). The restoration of the cytoplasm might be achieved by a series of implantations of new nuclei to remove inhibitory material from the injected cell cytoplasm.

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