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Change in compatibility after microinjection of cytoplasm in amoebae

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Summary. A cytoplasmic fraction from D32, a clone of amoebae derived from *Amoeba proteus* injected with cytoplasm from *A. discoides,* inhibited cell division in *A. proteus* but not in *A. discoides* indicating a permanent change with respect to compatibility.

When cytoplasm or cytoplasmic fractions of *Amoeba discoides* are microinjected into *A.proteus,* a percentage of the injected cells shows an incompatibility phenomenon, the inhibition of cell division[1]. Injected cells which are not inhibited divide normally and form viable clones. If these surviving clones are examined, many show changed characteristics such that they no longer resemble the original injected cells, i.e. *A.proteus,* but resemble the donor cells, i.e. *A.discoides*[2]. These characters include changed nuclear diameter and sensitivity to growth in antibiotics. We report another character change, an inhibition of division active against the original recipient strain.

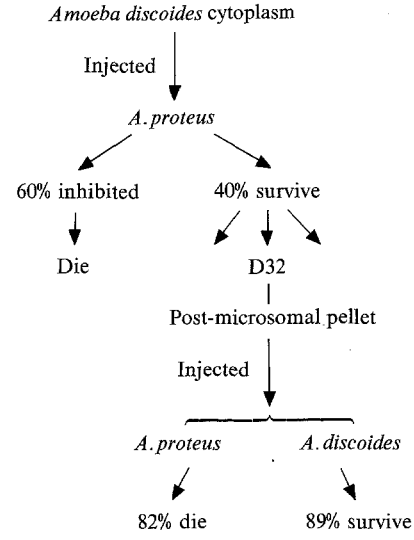
Materials and methods. Amoeba discoides, A.proteus and 'D32' were grown as 'wheat grain' cultures and *A.discoides* and D32 grown as mass cultures fed regularly on *Tetrahymena pyriformis*[2]. Cells from mass cultures were cleaned from food organisms, bulked and lightly homogenized in sucrose-TKM buffer (0.24 M sucrose, 0.05 M Tris-HCl, 0.025 M KCl, 0.005 M $MgCl_2$, pH 7.4)[2]. Nuclei, mitochondria and microsomes were removed by centrifugation, and the post-microsomal supernatant was centrifuged for 6 h at 105,000 × g to yield a post-microsomal pellet. This pellet was resuspended in 400 µl TKM buffer and stored at −20 °C in small aliquots. Amoebae were microinjected with small volumes of pellet using techniques described previously[3] and cells mainained as 'singles' until they divided, or died without division after 14 days.

Results and discussion. If cytoplasm from *A.discoides* is microinjected into *A.proteus,* 60% of injected cells fail to divide and die within 14 days. Fractionation of the cytoplasm by centrifugation showed that this 'inhibitory' activity was located in the post-microsomal supernatant and when injected into *A.proteus* led to inhibition of cell division in 90% of recipient cells[4]. Further high-speed centrifugation yielded a post-microsomal pellet which inhibited division in over 95% and sometimes 100% of injected cells[5]. The strain 'D32' was a clone derived from an *A.proteus* cell injected with whole cytoplasm from *A.discoides* which was not inhibited and produced a viable clone. When cells of D32 were examined, both mean nuclear diameter and sensitivity to growth in the antibiotics streptomycin, neomycin, erythromycin and chloroquine resembled those of *A.discoides*[2]. That the cytoplasmic environment of D32 had changed was suspected from results of nuclear transfer experiments, where 48% of transferred *A.discoides* nuclei survived in D32 cytoplasm, whereas less than 1% of transferred *A.discoides* nuclei survived in *A.proteus* cytoplasm. D32 was grown in mass culture and samples of the postmicrosomal pellet were prepared by centrifugation. The results obtained when some of the D32 pellet was injected into *A.proteus* and *A.discoides* are shown in the table and the figure together with the results obtained using a post-microsomal pellet prepared from *A.discoides* and *A.proteus.* The inhibitory molecules present in the *A.discoides* pellet and active against *A.proteus* had no activity against D32. Similarly D32 inhibitory molecules had little effect on the division of *A.discoides* but inhibited 82% of the injected *A.proteus.* Thus, not only had D32 changed with respect to mean nuclear diameter and response to antibiotics, it now had an inhibitory activity which resembled that of the donor *A.discoides.* These inhibitory molecules are protein together with 3% RNA[5,6] and this change of character would appear to be more complex than the changes previously reported. Since the D32 clone was obtained some years ago, this change in character is permanent and is further evidence of the presence in amoebae cytoplasm of molecules which carry genetic information and may be transferred by microinjection. Studies using polyacrylamide gel electrophoresis have shown that this genetic information resides in low molecular weight RNA molecules, but the mechanisms involved in information transfer are, as yet, unknown[7].

Compatibility of post-microsomal pellets in amoebae

Source of post-microsomal pellet	Strain injected	No. of cells injected	% division
D32*	*A.proteus*	72	18
D32	*A.discoides*	79	89
A.discoides	*A.proteus*	94	5
A.discoides	D32	75	98
A.proteus	*A.discoides*	90	5

* A clone of amoebae derived from an *A.proteus* cell injected with *A.discoides* cytoplasm.



Stages in the experiment.

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