lation of aminoisobutyrate uptake in uncloned H35 cells (*17*). Such sensitivity suggests mediation of the response through the insulin receptor. This in contrast to other reports in which the ability of insulin to act as a growth factor has been attributed to its acting through a receptor for an insulin-like growth factor, MSA (multiplication-stimulating activity) from the BRL-3A cell line (*18-20*). Both of these reports were substantiated by the observation that the F(ab) fragment of immunoglobulin G antibodies to the insulin receptor would not block insulin mitogenic activity in spite of its ability to displace iodinated insulin from its receptor (*18-20*) and to block insulin-stimulated glucose oxidation.

The significance of the ability of insulin to act as a potent growth factor is reinforced by early observations that insulin provokes an increase in hepatic DNA content, an effect that is attributable to increased DNA synthesis and not to a change in the ploidy of the cells (*21*). Also, investigators have reported the concomitant occurrence of insulin resistance in patients with growth disorders suggesting the possibility that the lesion responsible for the insulin resistance is a contributing factor in the associated growth defect (*22, 23*).

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24. This research was supported by grant AM 24047 from NIH to J.W.K. We especially thank W. D. Wicks in whose laboratory this work was conducted.

28 April 1980; revised 21 November 1980

Rapid Induction of Cellular Strain Specificity by Newly Acquired Cytoplasmic Components in Amoebas

Abstract. *A new strain-specific character was induced in amoebas by bacterial endosymbionts after only 10 to 15 cell generations of symbiosis. The nuclei of changed amoebas not only became incompatible with the cytoplasm of the original strain, but also exerted a strong lethal effect on intact amoebas of the same original strain.*

We previously reported bacterial endosymbionts of *Amoeba proteus* that changed from parasites (*1*) to required cytoplasmic components within a few years (*2*). These symbiotic bacteria are capable of infecting other amoebas and of causing the latter to become dependent on the symbionts in 200 cell generations (18 months) (*3*). We now report that within 4 weeks (10 to 15 generations) of infection with the symbiotic bacteria, host amoebas may become genetically distinct from the original strain.

Amoebas do not reproduce sexually, and the only way to test genetic compatibility between two strains is through nuclear transplantation. Exchange of nuclei between amoebas of the same strain (homotransplants) results in the formation of viable cells, whereas heterotransplants (that is, between amoebas of different strains) are nonviable because of nuclear-cytoplasmic incompatibility (*4*). Heterologous nuclei also exert a strong lethal effect when implanted into amoebas of different strains (*5*) or genera (*6*). Thus, when a heterokaryon is produced from different strains, both donor and host nuclei become nonviable within minutes, in contrast to homokaryons which produce viable clones. The lethal effect of heterologous nuclei has been attributed to a strain-specific lethal factor (*5*), which consists of high-molecular-weight proteins (*7*). Thus an internuclear lethal effect is one criterion for determining cell variation and strain specificity.

Taking advantage of the specificity of this phenomenon, we studied the role of newly acquired cell components, namely, endosymbionts of the xD strain of *A. proteus* and their ability to effect permanent cell changes in their hosts. The xD strain arose in 1966 after spontaneous infection of the D strain with rod-shaped X bacteria (*1*). The xD strain now requires these endosymbionts and its nuclei are not viable in their absence (*2*). Our studies were designed to determine (i) if the D and xD strains are sufficiently different to display nuclear incompatibility, (ii) how many cell generations are required before such incompatibility is expressed, and (iii) whether the lethal effect is dependent on the continued presence of the endosymbiotic bacteria. Changes in compatibility between D and xD amoebas are easily studied, since symbiosis between D amoebas and X bacteria can be established at will (*8*). We report here that xD amoeba nuclei are unilaterally lethal to D amoebas (that is, D nuclei are nonlethal to xD amoebas). Furthermore, the introduction of endosymbionts into a D amoeba induces irreversible changes in the host progeny in 10 to 15 generations (about 4 weeks), and these changes render the nuclei of new xD amoebas lethal to D amoebas when they are transplanted into these cells.

First, to test mutual lethal effects, we inserted nuclei of one strain into amoebas of the other by means of a de Fonbrune micromanipulator on agar-coated slides (*9*). After 5 minutes we removed the nuclei micrurgically. In previous studies, this period was sufficient for heterologous nuclei to exert lethal effects (*5*). Homokaryons were studied concurrently. The amoebas were cultured singly in watch glasses (U.S. Bureau of Plant Industry; Thomas, Philadelphia) containing modified Chalkley's medium (*2*), and we observed the amoebas until they either formed viable clones (five or more divisions) or died. The amoebas were fed three times a week with *Tetrahymena* (*10*) and kept at 20°C.

As shown in Table 1, 84.4 percent of the D hosts that had contained xD nuclei for 5 minutes died, whereas only 14.5 percent of the corresponding homokaryons died. The lethal effect exerted

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by xD nuclei after only a 5-minute stay in D amoebas was somewhat weaker than that exhibited by those that remained in D cells for 24 hours or longer (not shown in the table). However, we decided to remove heterologous nuclei after 5 minutes because it became impossible to distinguish them from the host nuclei after this time. Amoebas that failed to form viable clones showed symptoms of ensuing death [hybrid syndrome (*4*)]. In addition, their nuclei appeared abnormal because of clumping of nucleoli. The interval between the first appearance of these symptoms and cell death varied between 3 and 20 days. Over 85 percent of the progenies of $(D_n)D_{nc}$ homokaryons and $(D_n)xD_{nc}$ heterokaryons formed viable clones without showing these symptoms (Table 1), indicating that D nuclei had no adverse effect on either D or xD amoebas.

Next, we used newly infected amoebas (designated NxD) as the nuclear donors to determine how long it took for the nuclei of newly infected D amoebas to acquire the lethal effect. The majority of D nuclei became lethal to other D amoebas as early as 4 weeks after becoming infected with X bacteria (Table 2). Nuclei of xD amoebas in symbiosis for only 4 to 9 weeks exerted as strong a lethal effect as those from the original xD strain that had been in culture for 14 years.

We then asked whether nuclei from symbiont-depleted xD amoebas retained the lethal effect on D amoebas after losing their symbionts. Symbiotic bacteria disappeared if xD amoebas were kept for 7 days at 26.5°C (*3*), with amoeba nuclei assuming an abnormal appearance with clumped nucleoli (*11*). The symbiont-depleted amoebas were kept at 26.5°C for another 7 days before their nuclei were tested for lethal effects, to assure that the nuclei were free from the influence of symbiotic bacteria. Of the 44 D amoebas that had hosted heat-treated xD nuclei for 5 minutes, 20 divided once or more, but only five formed permanent clones (that is, 88.6 percent of the amoebas that had been exposed to xD nuclei died). Thus, xD nuclei that had been in the symbiont-free cytoplasm for 7 days retained their lethal effect on D amoebas, indicating that the nuclear change was permanent.

Since the nuclei of newly infected D amoebas became lethal to D amoebas in 4 weeks, it was of interest to see if these changed nuclei were still compatible with the original D cytoplasm. Thus, we back-transferred the nuclei of NxD amoebas that had been in symbiosis for 4 to 15 weeks into the cytoplasm of D amoebas (Table 3). Most of the amoebas with nuclear transplants (that is, NxD_nD_c) failed to form viable clones, indicating that the D nuclei became incompatible with D cytoplasm after reproducing in xD cytoplasm for 4 to 15 weeks. However, when symbionts were removed from these newly established xD amoebas by culturing them at 26.5°C, most of them continued to grow and form viable cultures, confirming earlier results (*3*). Thus, it appeared that the nuclei of newly established NxD_n amoebas did not become fully dependent on symbionts and were able to remain viable if the symbionts were removed gradually.

Our study of the interaction between D and xD strains of amoebas shows that (i) nuclei of xD amoebas exert a lethal effect when inserted into D cells, (ii) a newly established xD amoeba strain may acquire a lethal activity within 4 weeks, (iii) the xD nuclei retain a lethal effect even after their symbionts have been removed, and (iv) most of the nuclei of newly established xD amoebas are incompatible with the original D cytoplasm after a 4-week stay in cytoplasm containing X bacteria.

Establishment of the original xD strain of amoebas took several hundred cell generations (a few years) (*2*). Biologists who are used to regarding cellular changes on a long geologic time scale have considered this as an exceptional case of rapid symbiont integration (*12*). Our present data demonstrate that an even smaller number of generations is required for symbiont integration and cell divergence.

The mechanism whereby bacterial symbionts cause rapid changes in cellular strain specificity is not known, although the observed lethal effect of xD nuclei on D amoebas is similar in etiology to that previously described for other strains of amoebas (*5*). By inference, it is likely that xD amoebas produce a new protein—the lethal factor (*7*)—as a consequence of becoming symbiotic. Involvement of such a factor in the killing phenomenon is further supported by our study in which we were able to neutralize the lethal effect of xD nuclei by "washing" them for 5 minutes in the cytoplasm of D amoebas before testing

Table 1. Strain specificity of D and xD amoebas, as determined by the lethal effects of their nuclei. The subscripts n and c represent the nucleus and cytoplasm, respectively.

Strain combination*	Number of cells studied	Number of cells dividing	Number of clones obtained	Lethal effect of nuclei (%)
$(xD_n)D_{nc}$	128	50	20	84.4
$(D_n)D_{nc}$	131	121	112	14.5
$(xD_n)xD_{nc}$	56	51	48	14.3
$(D_n)xD_{nc}$	71	69	66	7.1

*The parentheses denote that the donor nuclei were removed micrurgically from the host cells after 5 minutes. The host amoebas contained their own nuclei.

Table 2. Lethal effect of newly obtained xD amoeba nuclei on D amoebas. The prefix N indicates a newly established amoeba strain.

Strain combination*	Time in symbiosis	Number of cells studied	Number of cells dividing	Number of clones obtained	Lethal effect of nuclei (%)
$(NxD_n)D_{nc}$	4 weeks	107	33	4	96.3
$(NxD_n)D_{nc}$	9 weeks	38	13	6	84.2
$(xD_n)D_{nc}$	14 years	128	50	20	84.4

*The parentheses denote that the donor nuclei were removed micrurgically from the host cells after 5 minutes. The host amoebas contained their own nuclei.

Table 3. Compatibility between nuclei of newly obtained xD amoebas and D amoeba cytoplasm (back-transfer).

Strain combination*	Time in symbiosis	Number of cells studied	Number of cells dividing	Cells forming clones	
				Number	Percentage
D_nD_c		27	26	26	96.3
NxD_nD_c	4 weeks	40	15	2	5.0
NxD_nD_c	15 weeks	22	1	1	4.5
xD_nD_c	14 years	84	6	1	1.2

*The donor nuclei were transplanted into enucleated cytoplasm, and left in the latter.

their ability to kill intact D amoebas. Eleven out of 13 D amoebas that hosted washed xD nuclei for 5 minutes remained viable, whereas none of the D amoebas in which xD nuclei had been washed formed clones.

The new strain-specific lethal factor could be synthesized by xD amoebas either as a result of acquiring new DNA templates or by altered expression of existing chromosomal genes. Further work is needed to distinguish between these possibilities, but the latter mechanism would be simpler in that products of symbiotic bacteria could effect an alteration of gene expression of amoebas without involving any transfer of their own DNA's. Such progressive changes in nuclear synthetic activities caused by cytoplasm occurs regularly during embryonic development of metazoans.

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25 July 1980; revised 5 November 1980

Tris(dichloropropyl)phosphate, a Mutagenic Flame Retardant: Frequent Occurrence in Human Seminal Plasma

Abstract. *Negative-chemical-ionization mass spectral screening of extracts of human seminal plasma has revealed a presence of a Cl_7 ion cluster at a mass-to-charge ratio* (m/z) *of 463 in a significant number of the samples examined (34 out of 123). Experiments with different gases used to generate the negative-chemical-ionization plasma indicated that the ion at* m/z *463 was a chloride adduct of a Cl_6 molecule with a mass of 428 daltons. Negative-chemical-ionization mass measurement with ions from the iodoform mass spectrum used as reference peaks gave a mass of 427.882 daltons; $C_9H_{15}O_4PCl_6$ has a molecular weight of 427.883. Extraction of polyurethane foam with toluene produced an extract that consistently gave a negative-chemical-ionization spectrum containing an intense Cl_7 ion at* m/z *463. The component producing this ion was isolated, and its proton nuclear magnetic resonance spectrum confirmed that it was tris(1,3-dichloro-2-propyl)phosphate, a mutagenic flame retardant. The negative-chemical-ionization screening evidence suggests that this flame retardant or its isomer tris(2,3-dichloro-1-propyl)phosphate, or both, are absorbed into the body from formulations in which they are used as flame retardants. Remedial action seems indicated to reduce human exposure to these compounds.*

Negative-chemical-ionization (NCI) mass spectral screening of minimally cleaned extracts of environmental substrates has been successful in detecting part-per-billion concentrations of substances that are oxidizing agents, alkylating agents, or both (*1*). Such NCI mass spectra can be used to selectively detect the presence of polychlorinated organic substances (*1*, *2*), phosphate and phosphothioate insecticides (*3*), polynuclear aromatic hydrocarbons (*4*), and brominated flame retardant metabolites (*5*). The technique of NCI mass spectrometry is directly analogous to gas chromatography with electron-capture detection, a major difference being that NCI mass spectrometry supplies molecularly specific information about the substances that are detected. The basis for the success of these techniques in detecting toxic substances stems from the fact that molecules that are oxidizing agents, alkylating agents, or both generally have positive electron affinities or significant anion affinities in the gas phase, or both. Biological molecules, particularly neutral lipids, which are among the most difficult substances to remove in residue cleanup procedures, generally have negative electron affinities and low NCI sensitivities. As a result, exceptionally simple cleanup procedures (*6*) can be used for NCI screening applications.

In a survey of toxic substances in seminal plasma (samples were obtained from student donors) as a function of sperm density (*7*) we frequently encountered (34 out of 123 cases) an isotope pattern indicating the presence of a cluster of seven chlorines (Cl_7) at a mass-to-charge ratio (*m/z*) of 463. The appearance of this isotope cluster was correlated with the appearance of a Cl_6 cluster at *m/z* 427 and a Cl_4 cluster at *m/z* 317. These correlations suggested that the ion cluster at *m/z* 463 was the result of the attachment of chloride to a molecule containing six chlorines with a molecular weight of 428. This presumption was strengthened when we obtained the NCI spectrum without a source of chloride, in which case only the ions at *m/z* 427 and 317 appeared. The ion at *m/z* 317 had previously been misassigned as the chloride adduct of DDMU [1-chloro-2,2-bis(*p*-chloro-phenyl)ethylene] (*6*).

Mass measurement of the ion cluster at *m/z* 463 in the negative ion mode with the chloride adduct of iodoform at *m/z* 428.691 used as a standard gave an exact mass of 462.851 daltons for the ^{35}Cl ion of the *m/z* 463 cluster. The elemental composition $C_9H_{15}O_4{}^{35}Cl_7P$ has an exact mass of 462.852 daltons.

A Cl_7 ion cluster at *m/z* 463 that corresponded in every way to the ion found in samples of human seminal plasma that had been cleaned up by steam distillation continuous liquid-liquid extraction (*6*, *7*) was a consistent occurrence in our attempts to obtain procedural blanks for a process designed to isolate planar polynuclear aromatics by adsorption on activated carbon supported on polyurethane foam. The spectra of these extracts also exhibited the ion clusters at *m/z* 427 (Cl_6) and 317 (Cl_4). As a result of this coincidence, we extracted the polyurethane foam with toluene in a Soxhlet extractor and isolated the component that gave rise to the spectrum by chromatography on alumina. The methylene chloride NCI mass spectrum of this component is illustrated in Fig. 1. Since milligram quantities of this material were available, it was possible to obtain a proton nuclear magnetic resonance (NMR) spectrum (Bruker 270-MHz Fourier transform NMR spectrometer) and a complete high-resolution, electron-impact mass spectrum. The proton spectrum of the component giving rise to the *m/z* 463 ion cluster is illustrated in Fig. 2. Proton decoupling experiments indicated that the resonances at 3.86 δ and 3.83 δ were

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