sumption of a potential of 70 mv across the membrane, 6.44 additional kilocalories would be required to transport 2 moles of Ca^{2+}, therefore exceeding the ΔG values of *p*-NPP and ATP. This suggests that either no electrical work is accomplished during Ca^{2+} transport (for example, equivalent negative charge produced on the inner side of the membrane, simultaneous to cation release), or no membrane electrical potential is formed in fragmented sarcoplasmic reticulum.

It appears then, that in appropriate experimental conditions the maximum filling capacity of the vesicles corresponds to a certain gradient ($Ca^{2+}{}_{in}$/$Ca^{2+}{}_{out}$), where the energy required for transport of 2 moles of Ca^{2+} matches ΔG of the substrate used in the hydrolytic reaction. Therefore, the active transport is energetically limited and near the asymptote the free energy efficiency approaches 100 percent. Furthermore, independent of the substrate structure, a ratio of a Ca^{2+} to substrate of 2 is maintained, and an identical amount of work is required for Ca^{2+} transport at a given gradient. In contrast, the structure of the substrate (quite different for *p*-NPP as opposed to ATP) has a profound influence on the reaction rates and the affinity of the system for Ca^{2+}. This can be easily demonstrated from a study of the linear rates of adenosine triphosphatase and *p*-nitrophenyl phosphatase catalyzed by Triton-solubilized SR (*11*), whose half-maximum activation is obtained in the presence of 6×10^{-7} and $7 \times 10^{-6} M$ Ca^{2+}, respectively.

As opposed to *p*-NPP, *p*-nitrophenyl acetate does not sustain appreciable activity, in spite of a high negative ΔG value. This suggests that the presence of a phosphate ester is necessary to substrate utilization for Ca^{2+} transport.

In view of the apparently identical mechanism of Ca^{2+} transport in the presence of *p*-NPP and ATP, it would be interesting to know whether hydrolysis of the two substrates is catalyzed by the same enzymatic site or whether preliminary phosphorylation of intermediates is required for utilization of different substrates. We know that both activities require Mg^{2+} and Ca^{2+} and that ATP is a competitive inhibitor of *p*-nitrophenyl phosphatase (Fig. 4). The fact that adenosine diphosphate (ADP) is also an inhibitor of *p*-nitrophenyl phosphatase indicates that intermediate phosphorylation of trace amounts of endogenous ADP is an improbable step in the mechanism of *p*-NPP hydrolysis catalyzed by SR. However, a definite answer to the identity of the enzyme or enzymes will only be obtained after solubilization and purification of the involved protein. At any rate, *p*-NPP is a convenient chromogenic substrate that, because of coupling of its relatively slow hydrolysis to Ca^{2+} transport, allows interesting kinetic and thermodynamic observations.

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5 August 1970; revised 21 October 1970 ■

Chromosome Lesions Produced with an Argon Laser Microbeam without Dye Sensitization

Abstract. *Improvements in the argon laser microbeam have made it possible to cause damage to chromosomes of tissue culture cells without prior treatment of the cells with a photosensitizing agent. These results have been confirmed independently in two laboratories.*

In previous studies of argon laser microirradiation it has been necessary to treat the cells with a photosensitizing agent (*1*). When an appropriate vital dye, such as acridine orange, is introduced into a tissue culture chamber for 5 minutes, the dye molecules intercalate into the DNA helix, making the chromosome (and specifically the DNA) light-sensitive. Subsequent irradiation of specific chromosomes with intense laser light has permitted physical deletion of selected portions of chromosomes, with concomitant functional loss. However, the necessity of using a vital dye increases the chance of secondary effects attributable to the dye itself, with the result that long-term experiments are difficult, if not impossible.

Improvements on the prototype argon laser microbeam system (*2*), as well as the construction of a new system in which a high-power argon laser is used (*3*), have permitted microirradiation with much more laser energy than before. This capability permits the production of discrete chromosomal lesions without the addition of a vital dye. These results have been confirmed independently in two separate laboratories.

The tissue culture techniques are similar to those described earlier (*1*, *4*). Primary explants of salamander (*Taricha*) lung are established in Rose multipurpose culture chambers (*5*). The culture medium is minimum essential Eagle's (*6*) medium with 10 percent fetal calf serum, penicillin, and streptomycin added. Mitotic chromosomes are clearly visible 1 to 3 weeks after establishment of the culture. Chromosomes are irradiated in prophase, metaphase, or anaphase.

The two microbeam systems consist of argon lasers combined with Zeiss photomicroscopes and closed circuit television receivers. In the system used at the Pasadena Foundation for Medical Research (*2*) a 1.5-watt (peak power per pulse, 25-μsec half pulse width) argon laser in multimode, multiline operation is used; in the system used at the University of Michigan (*3*) a 12-watt (peak power per pulse, 30-μsec half pulse width) argon laser in single mode, multiline operation is used. Energy measurements in the Michigan system are made with a calibrated vacuum photodiode (spectral response of 5), fitted with a narrow beam adapter, and

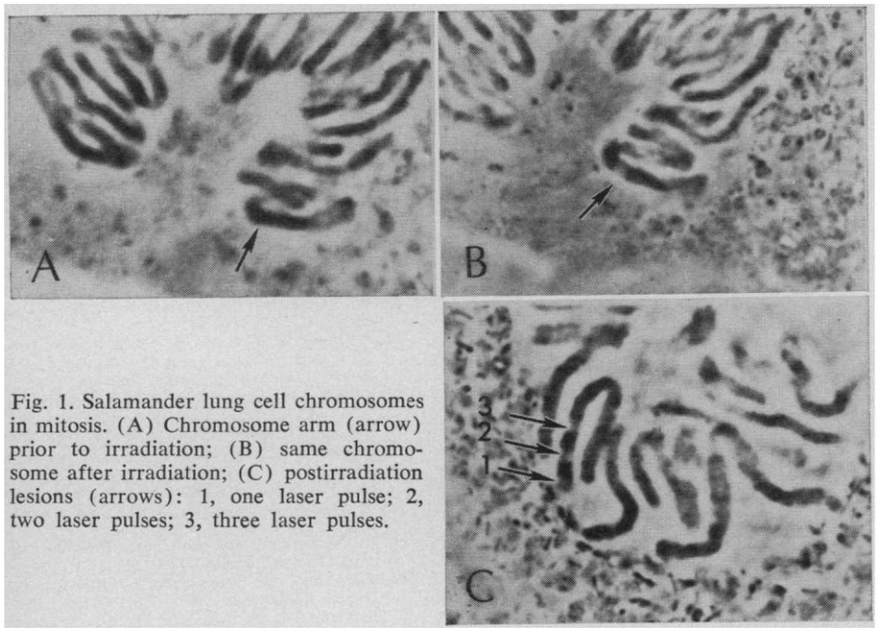

Fig. 1. Salamander lung cell chromosomes in mitosis. (A) Chromosome arm (arrow) prior to irradiation; (B) same chromosome after irradiation; (C) postirradiation lesions (arrows): 1, one laser pulse; 2, two laser pulses; 3, three laser pulses.

attached to a flash integrator system (International Light model 610/600). By focusing the oil immersion objective lens (Zeiss neofluar, × 100) of the photomicroscope directly onto the center of the narrow beam adapter, we determined that the system was 53 percent efficient. The energy at the focal point of the objective lens was 191 μjoule. The energy at the focal point of the Pasadena system is estimated to be approximately 30 μjoule. In our earlier microbeam studies (*1*) the energy at the focal point was approximately 10 to 15 μjoule.

Irradiation was accomplished by placing a culture chamber on the microscope stage and locating a mitotic cell on the television screen. A selected arm of a chromosome was moved under the cross hairs on the television screen, and the laser was fired. The results of single and multiple irradiations are illustrated in Fig. 1. In Fig. 1C lesions 1, 2, and 3, respectively, are the results of one, two, and three laser pulses at the same chromosomal site. In the case of one laser pulse, the lesion became evident 20 to 30 seconds after irradiation; two laser pulses resulted in a visible lesion 5 to 10 seconds after irradiation; three laser pulses resulted in a lesion that was immediately visible. Initially these results did not appear to be particularly significant because of the possibility of variation in the absorbing material along the chromosome. However, reports from Pasadena indicated that, with the 1.5-watt laser system, lesions did not become evident for 10 to 15 minutes after irradiation (*7*).

Chromosomal lesions produced in both laboratories with and without dye sensitization appear similar in the phase microscope. However, subsequent histochemical analysis suggests that the lesions produced with sensitization of the chromosomes by acridine orange are different from the lesions produced without the addition of the dye. The laser-induced lesions after treatment with acridine orange have been shown to react negatively to the Feulgen test (*1*). Feulgen staining of the nondye-treated chromosomes is positive throughout the area in which lesions caused by the laser occur. However, when the nondye-treated chromosomes are stained for basic protein with alkaline fast green, the lesion areas react negatively.

These results indicate that the DNA can be affected by laser irradiation after dye sensitization, or that the protein can be selectively affected by laser irradiation without dye sensitization. To further confirm these observations, we irradiated the nucleolus organizer regions of the chromosomes without previous dye sensitization. Even though visible "paling" lesions were observed in the nucleolus organizer regions, nucleoli were still synthesized by these regions. Previous experiments (*1*) with dye sensitization have demonstrated inactivation of the nucleolus organizer. A cytochemical and functional comparison of both types of lesions will be given elsewhere (*8*).

We can only speculate at this time about the nature of the chromosomal absorbance without dye sensitization. The cytochemical and functional data suggest that the absorbancy is associated with chromosomal protein. Several possibilities exist: (i) there is a slight natural absorbance by some protein moiety; (ii) the artificial conditions of tissue culture have, in effect, altered the protein sufficiently to precipitate a change in absorbance; (iii) the high photon density in the focused spot is causing a two-photon effect. The third alternative seems the least likely, since the two-photon effect would be expected to alter DNA as well as protein. The second alternative cannot be ruled out until sufficient control experiments are performed on chromosomes of cells not in tissue culture. The first alternative certainly seems possible, since, until laser sources became available, chromosomes had never been subjected to such intense visible light. It might therefore be possible that a slight absorbancy by nucleoproteins at the argon wavelength (488 or 514 nm) does exist, but merely that it has not been detectable with conventional photometric devices.

In addition to the above three mechanisms, other possibilities undoubtedly will be suggested. Notwithstanding, whichever mechanism is operational, the fact that either chromosomal DNA or protein can be selectively altered by laser microirradiation, either in combination with specific photosensitizing agents or without such agents, may prove to be a very useful comparative analytical method for the study of chromosome structure, function, and behavior.

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2. The original system, as described by us (*1*), had considerable loss of energy because only 50 percent of the laser energy entered the focusing objective lens. The addition of a lens with a 20-inch (50.8-cm) focal length between the laser and the microscope increased the amount of laser energy entering the objective lens of the microscope.
3. An argon laser (Hughes Aircraft model 3030H) is being used. This laser has a peak power of 35 watts in multimode operation and 12 watts in single mode operation. In addition, a prism wavelength selector permits the utiliza-

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23 October 1970; revised 30 November 1970 ■

Embryonic and Neoplastic Cell Surfaces: Availability of Receptors for Concanavalin A and Wheat Germ Agglutinin

Abstract. *Embryonic tissue cells dissociated with ethylenediaminetetraacetate are readily agglutinated by the carbohydrate-binding protein concanavalin A. In this property, they resemble transformed, neoplastic cells; and they differ from untransformed adult cells, which are agglutinated by concanavalin A only after their receptors are unmasked by proteolytic treatment. Receptor sites for wheat germ agglutinin are also present on the surface of embryonic cells, but in a masked form, as on untransformed adult culture cells; they can be unmasked by treatment of the cells with trypsin. Concanavalin A binding sites on embryonic cells may function in cell contact and cell organization during embryonic morphogenesis and differentiation and later become masked in adult cells. The unmasking of these sites in neoplastic cells may represent a return, in this respect, to a condition resembling that of embryonic cells and may be related to cell mobility associated with infiltration and metastasis.*

Changes in the properties of cell surfaces represent an important aspect of embryonic differentiation, morphogenesis, neoplastic transformation, and metastasis. Studies of the histogenetic reaggregation of dissociated cells in vitro and of the self-assembly of tissues from cell suspensions have contributed to the understanding of the surface properties of cells (*1*). Another valuable approach to this problem is the use of exogenous proteins that specifically bind to various carbohydrates on the cell surface and cause cell agglutination. Neoplastic cells (transformed by carcinogens or viruses) are agglutinated by a glycoprotein from wheat germ (*2*) and by concanavalin A (Con A), a jack-bean globulin (*3*); but these proteins do not agglutinate untransformed cells. Untransformed cells do possess binding sites for these agglutinins, but in a masked form; thus treatment of untransformed cells with trypsin renders them agglutinable by exposing the carbohydrate-containing binding sites (*2*, *3*). On the other hand, treatment of untransformed cells with ethylenediaminetetraacetate (EDTA, Versene) does not render them agglutinable with these proteins. These findings suggest that the surfaces of normal cells differ from those of neoplastic cells by the inaccessibility of their sites for interaction with the above agglutinins and by the exposure of such sites through treatment of the cells with trypsin, but not with EDTA.

The above agglutination experiments were performed with adult cells from established cell culture lines; to further test the validity of their conclusions, we examined in the present study the question of whether embryonic cells, isolated from normal tissues, resembled untransformed or transformed adult, cultured cells in their possession of masked or exposed surface receptors for Con A and wheat germ agglutinin (WGA). The cells used in these experiments were freshly obtained from neural retina and liver tissues of 10-day chick embryos by dissociating these tissues into suspensions either with trypsin or with EDTA. The trypsinization procedure yields single cell suspensions after 20 minutes of incubation (38°C) in a 0.5 percent to 1 percent solution of crystalline trypsin in calcium- and magnesium-free Tyrode solution (CMF) (*4*); the trypsinized cells were thoroughly washed with Tyrode solution containing deoxyribonuclease (5 μg/ml) (*5*). For dissociation with EDTA, tissue fragments were incubated for 40 minutes in CMF with 0.02 percent disodium EDTA, washed with CMF, and then disrupted by pipetting; the resulting mixture of cells and tissue fragments was filtered through sieves which allowed passage of only single cells and of small clumps of cells.

To test for agglutination, 0.2 ml of cell suspension (1.5 to 2.5 × 10^5 cells) prepared either with trypsin or with EDTA was added to Erlenmeyer flasks (25 ml) which contained 3 ml of Tyrode solution with deoxyribonuclease (5 μg/ml) and various concentrations of Con A (Calbiochem) or WGA (*6*). The flasks were immediately placed on a gyratory shaker (70 rev/min), at 38°C and the presence and size of agglutinates was checked at various times with a microscope.

Embryonic retina and liver cells dissociated by trypsin were agglutinated by Con A in the concentration range of 100 to 1000 μg/ml. The magnitude of the effect depended directly on the concentration of Con A. Agglutination began within 10 minutes after the start of incubation on the shaker, and the agglutinates reached maximum size within 30 minutes to 1 hour (Fig. 1). The largest of these agglutinates, obtained with the highest concentrations of Con A, contained 100 to 200 cells. Control cultures without Con A contained no agglutinated cells at any time; after 30 minutes of incubation, control cells had begun to aggregate histogenetically, as normally expected, and had formed clusters of 20 to 40 cells. However, these aggregates of normal cells were quite different in morphology and size from the agglutinates formed in Con A cultures. As in previous studies (*3*), the agglutination of cells by Con A was inhibited by α-methyl-D-glucopyranoside, a carbohydrate that strongly binds to Con A; it was not inhibited by *N*-acetylglucosamine.

To determine whether the agglutination by Con A of embryonic tissue cells dispersed by trypsin was due to unmasking by the protease of receptor sites for Con A, as in the case of adult tissue culture cells, embryonic tissue cells dispersed by EDTA were tested next. Con A at concentrations of 50 to 1000 μg/ml rapidly agglutinated EDTA-dissociated retina cells, even though they had not been exposed to trypsin. Moreover, the agglutination of the EDTA-dispersed cells was considerably more pronounced than that of trypsin-dissociated cells, in that the "lag period" before the onset of noticeable agglutination was shorter and the 30-minute agglutinates were many times larger and contained thousands of cells. Even though EDTA-dissociated embryonic cells tend to adhere to each other more rapidly than do trypsin-dissociated cells also in