EFFECTS OF LIGHT ON ELONGATION AND BRANCHING IN PEA ROOTS

JOHN G. TORREY

(WITH THREE FIGURES)

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Introduction

The characteristic morphology of the branched tap-root system of an adult plant is determined by the number and location of lateral root meristems initiated by the primary root during the course of its development. Much interest has centered around the identification of the specific factors responsible for the initiation of the cellular divisions leading to the precise formation of lateral root primordia. It was shown by THIMANN (**16**) and others that auxin plays a role in the initiation of lateral roots in the primary root of such plants as the garden pea, *Pisum sativum*. Evidence from recent studies of isolated pea roots grown in sterile nutrient medium (**17**) indicated that some other factor or factors act together with auxin to control lateral root formation in this plant. The continued study of these unknown factors revealed some remarkable effects of light on root growth—effects which may contribute to our understanding of the processes controlling root growth.

Relatively few investigators have described direct effects of illumination on the growth of this usually subterranean organ of the plant body. Scattered reports in the literature including such early work as that by DARWIN (**2**) and others (**7**), demonstrated an inhibitory effect of white light on root elongation. SEGELITZ (**15**) reported that isolated corn roots grown in culture in the dark extend more rapidly than roots exposed to white light, an effect attributed to the production of auxin by roots in the light. ROBBINS and MANEVAL (**14**) reported that the growth of excised corn roots was favorably influenced by light, an effect also reported by WHITE (**20**) for the excised roots of wheat grown in sterile culture. NAUNDORF (**8**) showed that illumination causes increased auxin production in the roots of intact or seedless Helianthus plants, which results in a slight acceleration of root elongation. According to Naundorf, auxin stimulation of root elongation may occur when suboptimal concentrations of auxin are normally present in the root. Phototropic responses by roots, either positive or negative, are recorded for a number of different species of plants and attempts have been made (**8**) to explain these responses in terms of differential auxin production in the light and dark.

WHITE (**21**) has stated that light has no observable effect on the growth of tomato roots in a sterile nutrient medium. ROBBINS (**13**) reported a peculiar periodic formation of root hairs by isolated roots of Datura grown in

sterile culture. Root hair formation occurred during daylight hours and was accompanied by a reduced rate of root elongation.

There are some reports on the effect of light on root initiation. WENT (**19**) studied the effect of different wave lengths of light on the initiation of adventitious roots in etiolated pea stem cuttings. He found that illumination at any wave length decreases lateral root initiation by decreasing the effectiveness of the applied indoleacetic acid (IAA) on the root initiation process. Recently, GALSTON and HAND (**5**) suggested that light decreases the efficiency of IAA in the initiation of roots in stem cuttings by stimulating the IAA-oxidizing system present in the etiolated pea stem tissue. No reports have been found on the effect of light on lateral root initiation in roots.

In recent years, the study of plant responses to light has been found increasingly more useful in the analysis of physiological and biochemical phenomena. The experiments reported here on the effect of light on the growth of the roots of intact seedlings and isolated roots were done as part of a general study of the unknown factors controlling lateral root formation in the growing plant.

Materials and methods

In all experiments, seeds of the garden pea, *Pisum sativum* variety Alaska, were used. The seeds were surface-sterilized in 0.1% mercury bichloride, rinsed in sterile water, and germinated in the dark in Petri dishes at 25° C for 48 to 72 hours. In studies with intact plants, seeds were transferred aseptically to a 250-ml. Erlenmeyer flask containing 50 ml. of nutrient medium (**1**), consisting of mineral salts, vitamins, sucrose, and 0.5% agar. When plant parts were used, excision of the desired parts was done aseptically, and the parts were transferred to Petri dishes containing 20 ml. of nutrient medium. Cultures were maintained at 25° C either in the dark or in the light. Continuous illumination was provided in a chamber lighted from above by six 20-watt Mazda fluorescent tubes, three daylight tubes and three white tubes. Illumination of 100 foot-candles was obtained at the treatment surface as measured with a Weston photronic exposure meter. Other light sources used in short-term illumination experiments included tungsten incandescent lamps with and without gelatin filters of known transmission. These light sources are specified in detail for the experiments described. Unless otherwise noted, all manipulations of plant materials subsequent to the initial sterilizing procedure were done under indirect illumination from a 10-watt, frosted, incandescent lamp screened by a blue, gelatin filter with maximum transmission at approximately 4400 to 4800 Å. This choice of safe light was made on the basis of certain experiments described below.

Throughout these experiments the morphological response of lateral root formation is used as an index of physiological activity in the study of the factors responsible for initiating cellular divisions in the pericycle of the

primary root axis. The response *per se* is conveniently expressed as the average number of lateral roots initiated per root with 10 to 16 plants per treatment. It was found convenient in handling a large number of plants to count only lateral roots which were 1 mm. or more in length. Lateral root primordia less than 1 mm. in length are therefore not included in the counts. In order to eliminate a possible source of error arising from light effects due to inhibition of lateral root elongation, total counts were made on cleared roots from each type of light experiment to be described. Whole roots were cleared in 2.5% NaOH and stained with safranin, and all lateral roots, including root primordia as well as macroscopically visible laterals, were counted under a dissecting microscope. Such counts indicated that while the total number of lateral roots thus determined was consistently greater than counts made of visible laterals, the increases were closely comparable in all series regardless of treatment. Thus, a macroscopic count of lateral roots longer than 1 mm. is a reliable index of rooting response.

Experimental results

The respective roles of shoot and cotyledons in seedling root development

In early experiments it was noted that the root systems of intact pea seedlings grown on agar nutrient medium in the light and in the dark were quite different. Lateral root formation was markedly reduced in plants grown in the light. To establish the respective roles of the shoot and the

TABLE I

ROOT ELONGATION AND LATERAL ROOT FORMATION BY INTACT PEA SEEDLINGS AND ISOLATED ROOTS WITH ATTACHED PLANT PARTS GROWN IN STERILE NUTRIENT MEDIUM IN THE LIGHT AND IN THE DARK.

Series number	Type plant	Seventh day			Fourteenth day		
		Average shoot length	Average root length	Laterals per root	Average shoot length	Average root length	Laterals per root
		mm.	*mm.*		*mm.*	*mm.*	
			Dark				
1	Intact seedling	131	130	21.5	380	216	49.0
2	Root plus cotyledons	....	133	24.7	....	209	48.0
3	Root plus shoot	35	84	0.0	60	116	2.6
4	Root only	....	79	0.0	....	129	9.8
			Light				
5	Intact seedling	23	101	7.9	86	209	9.0
6	Root plus cotyledons	....	85	7.2	....	164	8.0
7	Root plus shoot	14	91	0.0	25	141	0.0
8	Root only	....	45	0.0	....	77	0.0

cotyledons in the formation of the root system of peas grown under each condition, experiments were conducted with plants in which the shoot, the cotyledons, or both cotyledons and shoot were excised following the 48 hour germination period. Plants were then transferred to agar nutrient medium in flasks and grown in the light or in the dark for two weeks. The results of one such experiment are presented in table I. It is apparent that, under these conditions, the shoot has no effect in the light or dark on the formation of lateral roots. The cotyledons, however, must be present for normal lateral root formation to occur, as has been shown by RIPPEL (**12**). Substances essential to the initiation of lateral root primordia are furnished by

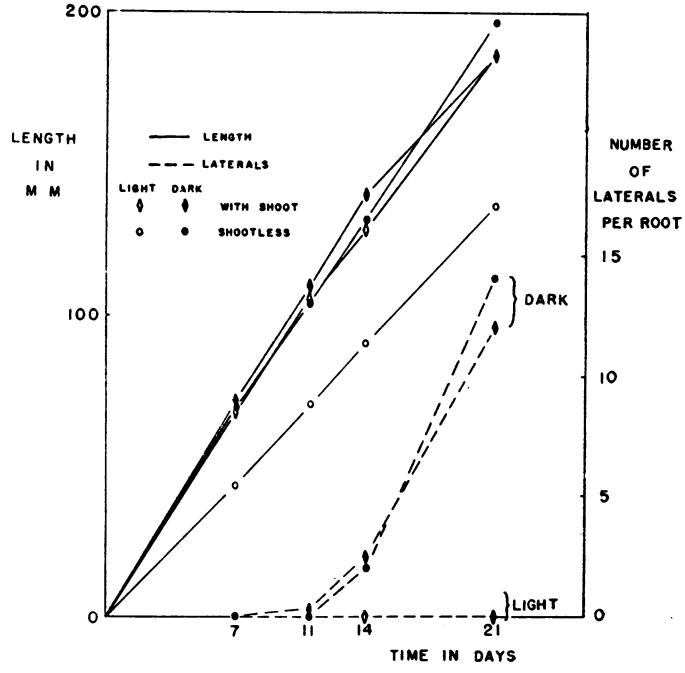

FIG. 1. Root elongation and lateral root formation in pea seedlings without cotyledons grown in white fluorescent light and in the dark.

the cotyledons and move into the primary root during the early stages of seedling growth. Attempts were made to substitute for the cotyledons by applying lanoline paste mixtures of IAA to the cotyledonary stumps. Such treatments had no effect on lateral root formation. In addition to auxin other substances apparently are provided by the cotyledons. Even in the presence of the cotyledons lateral root initiation is markedly inhibited by light.

To determine whether the shoot has any effect on lateral root formation during later stages of plant development, the cotyledons must be removed early during seedling ontogeny so as not to obscure any effect the shoot might exert. Pea seedlings without cotyledons, when cultured on agar nu-

trient medium in the dark, begin lateral root formation much later and at a slower rate than intact seedlings (Series 3, table I and fig. 1). In the dark, the presence or absence of the shoot has no effect on root initiation, as is shown in figure 1. Roots grown with or without the shoot produce 10 to 15 laterals per root in three weeks. Similar plants grown in the light do not form lateral roots at all. From the earlier experiments and the experiment summarized in figure 1, the following conclusions may be drawn: (a) the cotyledons are necessary for normal lateral root initiation in the intact pea seedling; (b) the shoot plays no measurable role in lateral root initiation whether or not the cotyledons are present; (c) in the absence of the cotyledons, lateral root initiation is delayed in plants grown in the dark, but will occur after a prolonged period of root elongation; and (d) lateral root initiation is inhibited in intact pea seedlings as well as cotyledonless plants by continuous white light.

Lateral root formation will be limited by the capacity of the roots to initiate lateral root primordia, a capacity which is determined by specific factors present within the primary root of the seedling. In the early stages of growth these factors are provided by the cotyledons; in later stages, the root appears to have the capacity to synthesize these factors or derive them from the nutrient medium. The experiments to follow were done with isolated roots, or excised roots with a single cotyledon attached, grown on agar nutrient medium in Petri dishes. The capacity of the isolated roots for the formation of lateral roots is initially limited by the substances already translocated into the root prior to the excision of the root tip; in roots with an attached cotyledon, the cotyledon provides a continuous supply of the factors necessary for lateral root formation.

The effect of light on roots with an attached cotyledon

A number of experiments were done to test the effect of duration of illumination on lateral root initiation. Seventy-two hours after germination,

TABLE II

EFFECT OF DURATION OF EXPOSURE TO LIGHT ON ROOT ELONGATION AND LATERAL ROOT FORMATION BY EXCISED PEA ROOTS WITH ATTACHED COTYLEDON.

Days of continuous light	Seventh day		Fourteenth day	
	Average root length	Laterals per root	Average root length	Laterals per root
	mm.		*mm.*	
0 (dark)	103	11.9	216	32.5
1	95	6.3	222	33.0
5	62	0.0	181	12.8
7	50	0.1	180	11.2
14	56	1.2	128	3.1
14 (red filter)	85	1.1	200	3.7

roots with a single attached cotyledon were transferred to nutrient medium in Petri dishes, two roots per dish, and placed under continuous illumination for a period of exposure varying from 1 to 14 days. After the illumination period, the roots were removed to a dark chamber until the total elapsed time was 14 days. In one series, a red filter (Corning Signal Red no. 2408 with transmission above 6100 Å) was interposed between the light source and the roots. The results, summarized in table II, indicate that during the period of illumination, lateral root initiation is almost completely inhibited (fig. 2 A). During the subsequent period of darkness, lateral root primordia

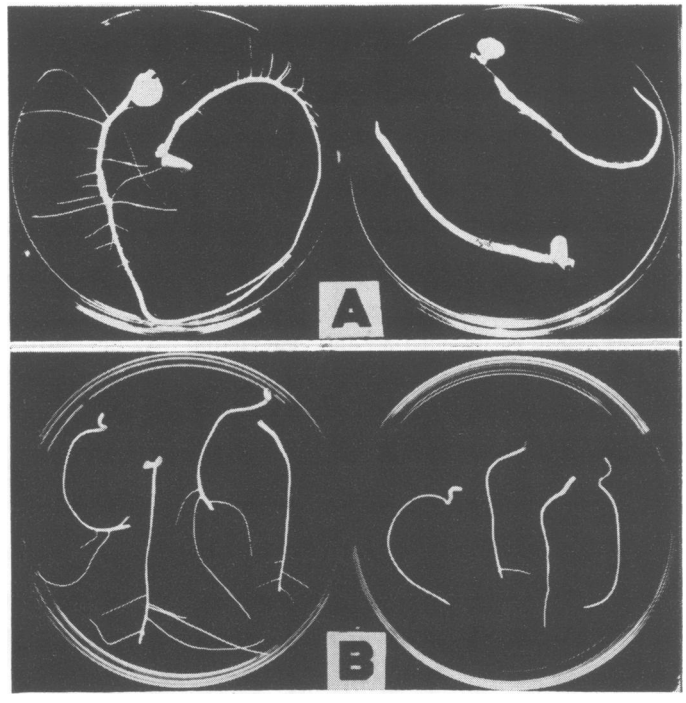

FIG. 2. A. Lateral root formation by isolated pea roots with attached cotyledon grown in sterile nutrient medium in the dark (left) and in continuous white fluorescent light (right). The photograph was made on the seventh day. B. Lateral root formation by decapitated pea roots grown in the dark (left) and in continuous white fluorescent light (right). The photograph was made one week after decapitation.

are initiated. It can be concluded that the light inactivates the factors necessary for lateral root initiation within the main axis of the root. Such light inactivation does not occur on exposing the cotyledon itself, either because of the dense nature of the cotyledon, or because the active substance is in a form which is unaffected by light. Upon movement of the factor into the primary root, inactivation occurs. It should also be noted that white light inhibits root elongation. The degree of inhibition is related to the duration of exposure. Of particular interest in this connection is the fact that red light with transmission in the range of 6100 to 7500 Å and of suffi-

ciently low intensity as to have little effect on root elongation is equally as effective as white light in the inhibition of lateral root initiation. Clearly, these two processes are separate phenomena, involving different factors which are either sensitive to different wave lengths of illumination or which, at a given wave length, show quite different sensitivities to the intensities tested.

An attempt was made to inhibit lateral root formation by exposure of roots to short periods of light daily in lieu of continuous illumination. A short period of incandescent white light was administered once every 24 hours to roots with attached cotyledons cultured in an agar nutrient medium. The data given in table III are averages from two experiments in which a 100-watt incandescent lamp was used at various distances. Measurements were made on the seventh day of the experiment. It is evident that a short exposure to white light once every 24 hours is effective in in-

TABLE III

RELATED EFFECTS OF DURATION AND INTENSITY OF ILLUMINATION ON ELONGATION AND LATERAL ROOT FORMATION OF EXCISED ROOTS WITH ATTACHED COTYLEDON. DAILY ILLUMINATION WITH UNFILTERED 100-WATT INCANDESCENT LAMP.

Exposure time in minutes	Distance from light	Approximate light intensity	Average root length	Laterals per root
	in.	*fc*	*mm.*	
None (dark)	....	....	112	15.9
1	12	120	90	4.9
4	12	120	89	4.6
8	12	120	90	3.3
1	48	5	90	5.9
4	48	5	90	5.4
8	48	5	91	5.6
1	96	1	104	6.7
4	96	1	104	7.2
8	96	1	103	7.1

hibiting lateral root initiation in these roots. The total illumination is considerably less in these treatments than in those using continuous fluorescent illumination. The increased effectiveness of incandescent light is presumably due to the increased intensities in the red range of the spectrum. Inspection of these results suggests the existence of a roughly reciprocal relationship between the light intensity and the duration of exposure in effectively causing lateral root inhibition.

Using the technique of repeated short light exposures, tests were run to determine the least inhibitory wave lengths. Roots with an attached cotyledon were illuminated one minute daily for six days, using a 100-watt incandescent lamp and gelatin filters of known transmission. As no means were available for the measurement of the absolute energies involved, the relative effectiveness of the different filters was difficult to assess. It was clear in all experiments, however, that red and yellow light are as effective

as white light in causing the inhibition. Blue and green light at approximately the same intensity inhibit lateral root formation much less effectively than the other wave lengths tested. On the basis of such experiments, the blue filter was chosen for safe light in all experimental work.

The effect of light on lateral root initiation in isolated roots

Continuous illumination or repeated exposure to light was necessary to prevent lateral root initiation in roots with an attached cotyledon since there was a continuous movement of root-forming factors from the cotyledon; however, with isolated roots in culture, a single short exposure to light was effective in inhibiting lateral root development. A report has already been made (**17**) on the quantitative response, in terms of lateral roots produced, exhibited by isolated roots which have been decapitated at the end of the first week of growth in nutrient culture. An account of the effect of light on the root-forming capacity of such decapitated roots follows.

TABLE IV

EFFECT OF SINGLE PERIODS OF ILLUMINATION ON LATERAL ROOT FORMATION BY DECAPITATED ROOTS GROWN IN STERILE NUTRIENT MEDIUM. FLUORESCENT LIGHT TREATMENT BEGUN AT TIME OF DECAPITATION.

Minutes of illumination	Average root length	Number of laterals on 10 roots
	mm.	
None (dark)	42.4	47
1	40.3	28
10	39.4	26
100	41.3	14
3000	40.8	14
5760	46.3	10
10080 (continuous)	45.0	5

Root tips excised from pea seeds germinated 48 hours were grown in Petri dishes in nutrient medium in the dark for one week by which time they had attained an average length of about 50 mm. After removal of 10 mm. of the tip, the bases were continued in the same medium in the dark for another week. At this time, 35 to 40 lateral roots were apparent on each 10 root bases in the region of the cut end. Decapitated roots maintained in continuous white fluorescent light produced almost no lateral roots (fig. 2 B). In the study of this light effect, root bases were exposed to single periods of light immediately following decapitation for periods varying from one minute to continuous exposure for seven days. Measurements were made one week after decapitation. The data from a typical experiment are presented in table IV, and the results of several such experiments are summarized in figure 3. The latter shows the relationship between duration of exposure and the inhibition of lateral root formation.

The most effective period for inhibition is the initial period of illumination. Ten minutes of exposure to light of 100 fc intensity causes a 50% inhibition of the lateral root formation which would occur in the dark. Additional periods of exposure to light are progressively less effective in causing inhibition of the process of root initiation. Results of experiments in which, after decapitation, the time of exposure to light was delayed for various periods indicate that the most effective time for illumination by white light is during the 24 hour period immediately following decapitation. Brief illumination 48 hours after decapitation is almost non-inhibitory since the root initiation phase has already occurred. It was also found that red light was equally as effective as unfiltered white light while blue light was

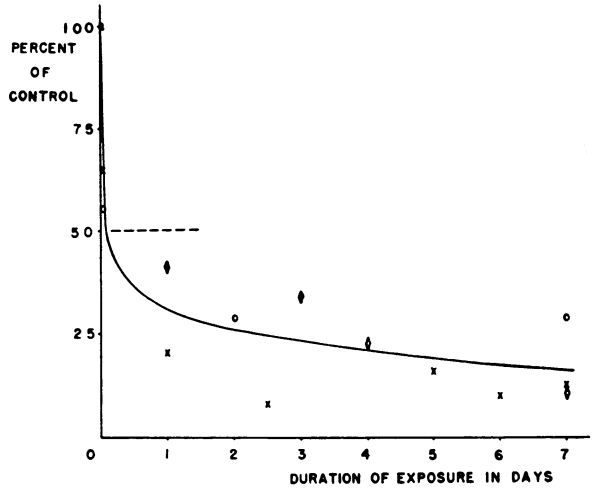

FIG. 3. The effect of duration of exposure to continuous white fluorescent light on lateral root formation by decapitated pea roots. Each point based on an average of 10 roots; data from three experiments included. 100% = dark control.

the least effective in causing inhibition of lateral root formation. The addition of IAA to the nutrient medium (1 mg./l.) following illumination had no effect on lateral root formation.

Discussion

The data presented here give evidence that absorption of radiant energy by pea roots causes inactivation of a substance present within the roots which is necessary for the initiation of cell divisions giving rise to lateral root primordia. In view of the evidence for a light-stimulated oxidation of auxin in plant tissues (**3**), it was thought possible that the inhibition of lateral root initiation was caused by light inactivation of auxin, one of the factors known to be involved in lateral root formation in pea roots; however, the addition of IAA to roots in the dark after illumination had no

effect on lateral root formation. Furthermore, the fact that red light was equally as effective as white light in the inactivation strongly suggests that auxin probably is not the factor directly affected by light. Auxin inactivation *in vitro* by a brei of etiolated pea epicotyls is greatest in the blue-green wave lengths (3900 to 5200 Å), the range of absorption of β-carotene and riboflavin (**4, 18**).

The experimental material should be good subject material for an action spectrum determination. Small amounts of illumination at relatively low intensities cause almost complete inhibition. The number of lateral roots produced is constant for equal total energies, and a reciprocal relationship is evident between the intensity of illumination and the duration of exposure. The results of a number of simple experiments with gelatin filters of known transmission at a given intensity indicate that the most effective wave lengths for inhibition of lateral root formation are within the red range of the spectrum (6100 to 7500 Å) and the least effective are in the blue-green region (4200 to 5400 Å). Although no definite statement concerning the effective wave lengths can be made until a precise determination of an action spectrum has been made, the evidence presented is of interest in view of the numerous recent reports of the spectral sensitivities for photoperiodic control of floral initiation in short and long-day plants (**9, 10**), for leaf and stem growth of etiolated pea seedlings (**11**) and for certain phases of inhibition of growth of the Avena internode (**6**). It is of interest that not only the etiolated and green stem tissues, but the root tissues as well, show morphological responses to illumination of apparently similar spectral distribution. The action spectrum reported by GOODWIN and OWENS (**6**) for 10% inhibition of internode growth is similar to that given for control of floral initiation (**9, 10**) and is considered an action curve for inhibition of cell division, the same response dealt with in the present experiments. Evidence concerning the nature of the light-absorbing pigment as well as the substances necessary for lateral root initiation may be forthcoming when a more precise knowledge of the action spectrum of this response has been achieved.

Although a detailed analysis of the effect of light on root elongation was not included in this study, some interesting observations on this point are worth noting. The reports in the literature on the influence of light on root elongation are numerous and often conflicting. That no single generalization can be made which will describe the effect of light on root elongation in all species of plants is clear from the review by HUBERT and FUNKE (**7**). The suggestion by NAUNDORF (**8**) that auxin production in the light determines the root response may lead to an ultimate explanation of the varying responses to white light shown by roots of different species of plants. According to THIMANN (**16**) the response of a root to an additional supply of auxin is greatly affected by the auxin concentration already present within the root. The response to auxin has been shown to vary widely from one species to another.

In the experiments reported here, the elongation of isolated roots is markedly inhibited by white light, whereas roots with the shoot attached, but lacking cotyledons, have a rate of elongation equal to those grown in the dark (table I and fig. 1). In this instance auxin production by the shoot may be involved. Since root elongation in low intensities of red light is closely comparable to the elongation of roots grown in the dark, even though lateral root formation is inhibited, it is suggested that auxin concentration in the root may be affected by white light. A careful study of the effect of light on root elongation might contribute to our understanding of the mechanism of root elongation.

Summary

Root elongation and lateral root formation in pea seedlings and isolated pea roots grown in a nutrient medium were studied under various conditions of artificial illumination. In seedlings, removal of the shoot has no effect on lateral root initiation whereas excision of the cotyledons stops lateral root formation under all light conditions. Maximum root elongation and lateral root formation occur when roots are maintained in the dark. White light apparently inactivates substances moving from the cotyledons which are essential for lateral root formation.

Excised roots with an attached cotyledon require continuous illumination or short exposure to light daily to effectively prevent lateral root initiation. Lateral root formation induced in cultured isolated roots by decapitation can be inhibited by a single short exposure to white light. By the use of gelatin filters, it was shown that red light inhibits lateral root formation much more effectively than filtered blue or green light. It is suggested that substances other than auxin are inactivated within the primary root by illumination.

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DEPARTMENT OF BOTANY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

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