Table 3. Linkage of *ksg* in conjugation. The donor and recipient strains are listed separately to aid in interpretation of the data. Matings were interrupted at 60 minutes in the selections for ArgG$^+$ PurC$^+$ and Leu$^+$ PurC$^+$, and at 120 minutes for His$^+$ PurC$^+$. Recombinants were purified once before being placed on appropriate mediums for scoring the phenotype.

Donor FS54	*argG*$^+$	*str*	*malA*$^+$	*leu*$^+$	(*ksg-3*)	*his*$^+$	*purC*
Recipient JC411	*argG*	*str*	*malA*	*leu*	(*ksg*$^+$)	*his*	*purC*$^+$

Experimental results

Phenotype selected	Scored (No.)	Ksg^R (No.)	Ksg^R (%)	Comment
ArgG$^+$ PurC$^+$	120	6	5	4 Leu$^+$; 4 Ksg^R; 0 Ksg^S
Leu$^+$ PurC$^+$	120	86	72	
His$^+$ PurC$^+$	178	78	43	60 Leu$^+$; 50 Ksg^R; 10 Ksg^S 118 Leu$^-$; 28 Ksg^R; 90 Ksg^S

Ksg^R, compared with 72 percent of those selected for Leu+. Since *his* is distal to *leu*, these data show that *ksg* is closer to *leu* than to *his*. Moreover, the recombinants selected for His+ were more often Ksg^R (78/178) than Leu+ (60/178); hence *ksg* appears to lie between *leu* and *his* (that is, clockwise to *leu*). Finally, in this cross the proposed sequence *leu-ksg-his* would require two recombinational exchanges to produce Leu− Ksg^R His+ and four exchanges to produce Leu+ Ksg^S His+; whereas the crossover requirements would be reversed if the sequence were *ksg-leu-his*. The proposed sequence is therefore further supported by the finding that the 178 His+ recombinants included 28 Leu− Ksg^R but only 10 Leu+ Ksg^S (Table 3).

The location of *ksg-3* was confirmed by a cross with HfrH, which donates clockwise with *leu* as its earliest marker and *lac* 10 minutes later. This *leu*+ *ksg*+ *lac*+ *str*+ strain was mated for 30 minutes with a *leu ksg-3 lac str* recipient (FS40). Of 60 Leu+ Str^R recombinants, 40 had received the donor Ksg^S marker, but only 2 had also received the donor Lac+ marker. Thus, *ksg-3* lies closer to *leu* than to *lac*.

Though further experiments are required to map the location of *ksg-3* precisely, it is clear that mutations which affect the 30*S* subunit do not all map in a single cluster. This finding may be important for our understanding of the control of the synthesis of 30*S* ribosomal components.

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Regenerative Calcium Release within Muscle Cells

Abstract. *Free calcium appears to trigger the release of stored calcium from the sarcoplasmic reticulum of skinned skeletal muscle fibers immersed in solutions with a low concentration of magnesium ion.*

There is considerable evidence that skeletal muscle myofilaments are activated by calcium (*1*) which is released into the surrounding space by the sarcoplasmic reticulum (*2*). The process is initiated physiologically by a decrease in the electrical potential across the surface membrane (*3*), but the mechanism of this release is poorly understood. The experiments reported here indicate that the release process has the capacity to be regenerative. This conclusion is based on an analysis of the contractions induced when fibers without their outer membranes are immersed in solutions of varied composition.

Single fibers from the semitendinosus muscle of the frog *Rana pipiens* were isolated in silicone oil (*4*), and the surface membrane was removed (*5*) to allow externally applied solutions free access to the myofilament space. Segments of these "skinned" fibers (1 to 3 mm long) were mounted in a force-measuring apparatus (*6*) before they were immersed in aqueous solutions (*7*). Low concentrations of calcium ion in the medium were controlled with ethylene glycol bis(aminoethylether)-*N*,*N*′-tetraacetic acid (EGTA), a calcium chelator with relatively little affinity for magnesium (*8*). Low concentrations of magnesium ion were established when the concentration of the metal was less than that of adenosine triphosphate (ATP) with which it forms a complex (*8*).

Skinned fibers immersed in solutions containing calcium buffered with EGTA slowly accumulate calcium for many seconds before developing force (*9*), and they retain this calcium when transferred to solutions of low concentrations of EGTA. Segments could therefore be loaded with calcium by immersion in a buffered calcium solution and then rinsed free of buffer in EGTA solutions. After this preparation, fibers that were exposed to unbuffered, "free" calcium solutions produced a quick contraction which was superimposed on a much slower contraction (Fig. 1A). The quick contraction could be interrupted by transferring the fiber to a solution containing a high concentration of EGTA (Fig. 1B); it did not depend on the major anion of the bathing medium, as it occurred equally well in solutions of potassium propionate and potassium chloride. The respones required that the fibers be loaded in a buffered calcium solution and that the concentration of free magnesium be relatively low. When fibers were immersed directly in the free calcium without exposure to the buffered calcium (Fig. 1C), or when the concentration of magnesium in solution exceeded that of ATP by 1 m*M*, quick contractions did not occur.

A quick contraction was also in-

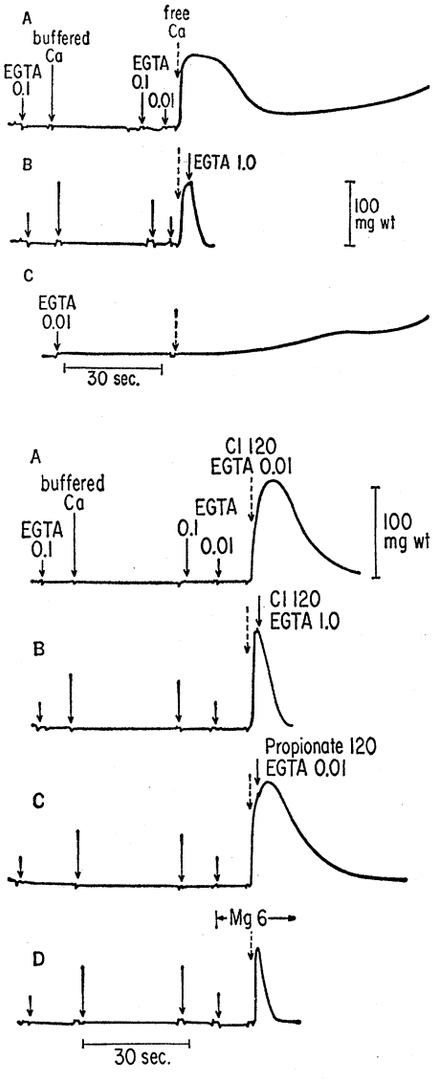

Fig. 1 (top left). Calcium-induced contractions of skinned muscle fibers. Arrows mark immersion in solutions containing buffered calcium, free calcium, or EGTA in the millimolar concentrations specified. (A) Quick contraction elicited by free calcium after loading. (B) Interruption of quick contraction by high concentration of EGTA. (C) Absence of quick contraction in unloaded fiber.

Fig. 2 (bottom left). Chloride-induced contractions of skinned muscle fibers. Arrows indicate solution changes; Cl 120 solutions contained 120 m*M* chloride in place of propionate, and Mg 6 solutions contained 6 m*M* $MgCl_2$ in place of 1 m*M* $MgCl_2$. All fibers were loaded with calcium before exposure to chloride. (A) Quick contraction elicited by chloride. (B) Interruption of contraction by high concentration of EGTA. (C) Course of contraction not influenced by subsequent removal of chloride. (D) Attenuated contraction obtained in the presence of 6 m*M* magnesium (1 m*M* ionized magnesium plus 5 m*M* MgATP).

duced when fibers that had been loaded in a buffered calcium solution containing propionate as the major anion were exposed to a high concentration of chloride (Fig. 2A). This contraction could also be curtailed by EGTA (Fig. 2B), but once initiated, it was not influenced by removing the chloride (Fig. 2C). Again, the response depended on the concentration of magnesium ion and required that the fibers be loaded in the buffered calcium. When the concentration of magnesium exceeded that of ATP, fibers generated only a brief twitch-like contraction (Fig. 2D). Fibers placed directly in the chloride solution without prior exposure to buffered calcium did not develop measurable tension.

Both contractions described above could be terminated by EGTA, could be induced only after exposure to buffered calcium, and were followed by relaxation without further change in the external medium. This suggests that both were due to a transient rise in the concentration of calcium in the space that contains the myofilaments, and that this calcium was derived from an internal source which had been preloaded. The contraction produced by a sudden rise in the concentration of chloride is believed to result from a change in electrical potential across the internal membranes (*10*). The contraction induced by calcium, on the other hand, seems to be initiated by a reaction of this ion with the internal membranes, for the response occurred in either chloride or propionate and was elicited by concentration changes of only $10^{-4}M$. Free calcium in the myofilament space therefore appears to trigger the release of internally stored calcium.

In the presence of free magnesium (1 m*M*), the contraction induced by calcium was abolished, whereas a brief twitch could still be induced by chloride. This suggests that a small quantity of calcium is released by a change in the electrical potential across the internal membranes, and when the concentration of magnesium ion is low, this calcium induces a larger liberation of calcium from internal stores. Thus the activation initiated by a change in electrical potential in this preparation appears to be amplified by a regenerative release of calcium.

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Simian Virus 40 in Polio Vaccine: Follow-Up of Newborn Recipients

Abstract. *Soon after birth, when susceptibility to carcinogens should be enhanced, a group of children received oral polio vaccine which was later found to contain significant amounts of simian virus 40. Eight years after the incident, no cancer deaths have been observed among the vaccinated children, but continued surveillance is needed before concluding that simian virus 40 is innocuous to man.*

The induction of cancer in laboratory animals by simian virus 40 (SV 40) (*1*) has had unusual public health implications. As an unrecognized contaminant of virus vaccines prepared in monkey kidney cell cultures prior to 1962 (*1*), SV 40 was given inadvertently with poliomyelitis and adenovirus vaccines to a substantial number of persons. The possibility that SV 40 is oncogenic in man was further suggested by its capacity to cause subclinical infection when administered with either attenuated (Sabin) or inactivated (Salk) polio vaccines (*2*) and by its capacity to produce cellular transformations suggestive of neoplastic growth in human