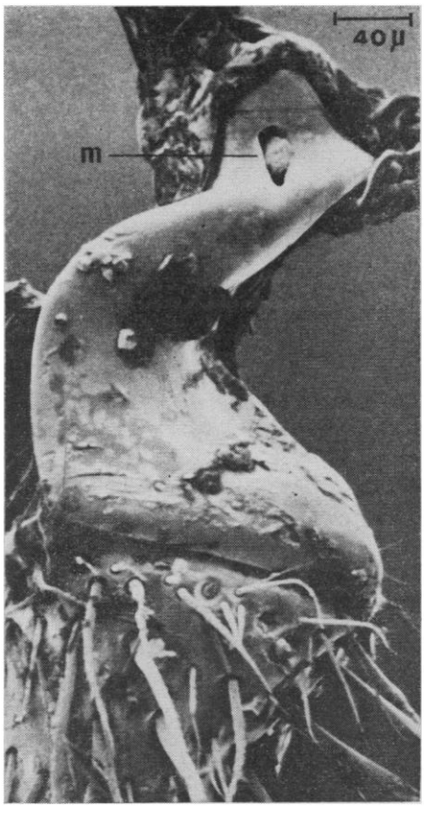

Fig. 1. Scanning electron micrograph of a cardo of *Dendroctonus ponderosae*. Mycangium (*m*) near proximal end with mycelium and spores of a blue-stain fungus or yeast, or both, in the opening.

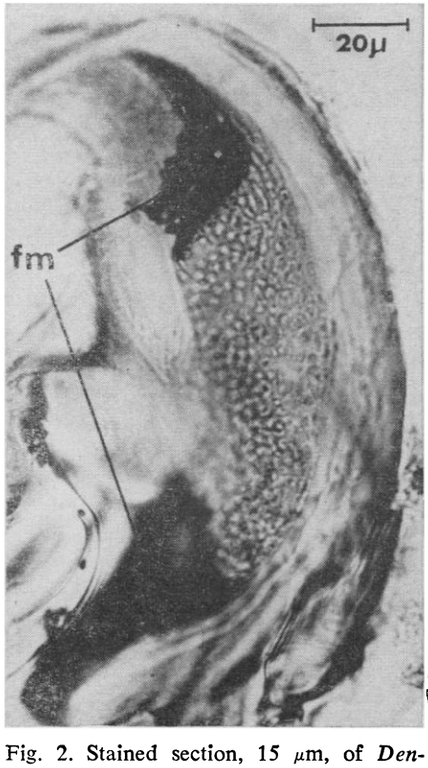

Fig. 2. Stained section, 15 μm, of *Dendroctonus ponderosae* mycangium showing reticulate structure. Fungus material (*fm*) is darkly stained.

[mostly *Pichia pini* (Holst) Phaff, *Hansenula capsulata* Wickerham, or *H. holstii* Wickerham]; *Trichoderma* spp., 4; *Penicillium* spp., 3; and *Cladosporium* spp., 2. Three mycangia yielded yellow bacterial colonies only, and two did not yield any microorganisms. Some mycangia yielding blue-stain fungi also yielded one, and sometimes two, yeasts. However, the two blue-stain fungi, *C. montia* and *E. clavigerum*, were never found together in the same mycangium, and the nonblue-stain fungi were usually solitary.

Mycelia and spores were observed but not cultured in 59 (48 females, 11 males) of 72 additional insects examined. However, when two of the 13 showing no visual evidence were cultured, both yielded *Ceratocystis montia* and a yeast. Of 29 *Dendroctonus ponderosae* adults sectioned and stained, fungi were present in the mycangia of 17 of 22 females and 5 of 7 males. The maxillae of 9 adult females examined in stained whole mounts all showed fungus material in the mycangia.

The variable length of fungal columns extending from the mycangia, the absence of microorganisms from some mycangia, and the occasional presence of fungi other than blue-stain or yeasts in other mycangia suggested that the microorganisms in the mycangium undergo change.

Symbiosis between certain fungi and wood and bark-inhabiting beetles of the family Scolytidae has been recognized for over 100 years (*8*). Discovery of a mycangium containing blue-stain fungi and yeasts in *D. ponderosae* indicates symbiosis between one or more of these microorganisms and this insect.

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References and Notes

1. M. Nunberg, *Ann. Mus. Zool. Polon.* **14**, 261 (1951); H. Francke-Grosmann, in *Symbiosis*, S. M. Henry Ed. (Academic Press, New York, 1967), vol. 2; L. R. Batra, *Trans. Kans. Acad. Sci.* **66**, 213 (1963).
2. S. H. Farris, *Can. Dep. For. Bimon. Prog. Rep. 21* (1965), p. 3.
3. S. H. Farris and A. Funk, *Can. Entomol.* **97**, 527 (1965).
4. "Stereoscan"—Cambridge Instrument Company, England.
5. A. D. Hopkins, *U.S. Dep. Agr. Bur. Entomol. Tech. Ser.* **17**, part 1 (1909).
6. H. Francke-Grosmann, *Verhandl. Deut. Ges. Angew. Entomol.* **14**, 139 (1957); L. P. Abrahamson, H. Chu, D. M. Norris, *Ann. Entomol. Soc. Amer.* **60**, 1107 (1967).
7. R. C. Robinson, *Can. J. Bot.* **40**, 609 (1962).
8. K. Graham, *Annu. Rev. Entomol.* **12**, 105 (1967).
9. We thank R. D. Mills (Civil Engineering Department, University of Calgary, Atla.) for the use of the scanning electron microscope, and J. Bloor of the same department for coating and photographing our specimens. We are grateful to the Department of Food Science and Technology, University of California, Davis, for identification of yeast cultures. J. Chapman of the Victoria Research Laboratory gave valuable advice during the study.

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Potassium Ions Asymmetrically Activate Erythrocyte Membrane Phosphatase

Abstract. *In reconstituted ghosts of human red cells, the cell membrane phosphatase is activated by external, but not by internal, potassium. This finding is consistent with the possible participation of the membrane phosphatase in the enzymatic system responsible for cation transport.*

The hydrolysis of adenosine triphosphate (ATP) by the cell membrane ($Na^+ + K^+$)-adenosine triphosphatase system seems to follow a sequence of at least two steps: (i) A ($Na^+ + Mg^{2+}$)-dependent transference of the terminal phosphate of ATP to the protein of the enzyme, and (ii) a K^+-activated release of inorganic phosphate from the phosphorylated protein. This sequence of reactions can be written as follows.

$$\text{ATP} + \text{enzyme} \xrightleftharpoons[Mg^{2+}]{Na^+} \text{enzyme-P} + \text{ADP}$$

$$\text{enzyme-P} + H_2O \xrightarrow{K^+} \text{enzyme} + P_i$$

It has been suggested that the membrane-bound *p*-nitrophenyl phosphatase activity, activated by K^+ and sensitive to ouabain, is the expression of the ability of the ($Na^+ + K^+$)-adenosine triphosphatase system to hydrolyze other phosphate esters apart from the phosphorylated intermediate (enzyme-P) (*1*). If the suggested identity of the K^+-activated phosphatase activity with the last step in the adenosine triphosphatase reaction is true, both enzymes would share the same K^+ sites. One of the main predictions that this assumption leads to is that the activation of the phosphatase by K^+ would take place, as in the ($Na^+ + K^+$)-adenosine triphosphatase system, only when K^+ is at the external surface of the cell membrane (*2*). We report experiments which test this prediction on "reconstituted ghosts" of human red cells.

The red cell membrane is highly permeable to the phosphatase substrate

p-nitrophenyl phosphate; and the membrane phosphatase activity which is sensitive to ouabain requires the internal presence of its substrate (*3*). Also, reconstituted ghosts (*4*), even when red cells are hemolyzed in 500 volumes of hypotonic solution, retain enough of the intracellular phosphatase to mask any contribution from the membrane phosphatase (*3*). We prepared reconstituted ghosts containing very low amounts of the intracellular phosphatase by hemolyzing the cells at 0°C in 300 volumes of hypotonic solution and allowing the hemolyzate to stand at this temperature for 10 minutes (*5*). This procedure allows the intracellular phosphatase activity to almost reach equilibrium with the hemolyzing solution.

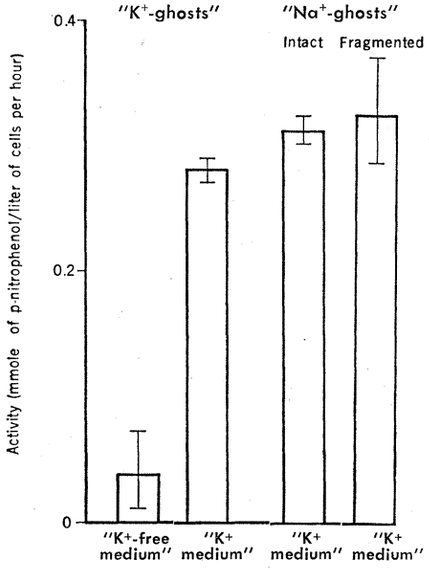

Fig. 1. Ouabain-sensitive hydrolysis (difference between rate of hydrolysis in absence and presence of $10^{-3}M$ ouabain) of *p*-nitrophenyl phosphate in reconstituted ghosts of red cells and in ghosts fragmented by freezing and thawing. The composition of the "K+-ghosts" before sealing was (mmole/liter): KCl, 50; $MgCl_2$, 5; NaCl, 10; and tris(hydroxymethyl)aminomethane (tris) hydrochloride, 85 (*p*H 7.8). The composition of the "Na+-ghosts" was, before sealing (mmole/liter): NaCl, 110; $MgCl_2$, 5; and tris-HCl, 40 (*p*H 7.8). The "K+-medium" contained: in the "K+-ghosts" experiments (mmole/liter), KCl, 100; NaCl, 30; $MgCl_2$, 10; and tris-HCl, 20 (*p*H 7.8); and in the "Na+-ghosts" experiments (mmole/liter), KCl, 50; choline-Cl, 90; $MgCl_2$, 10; and tris-HCl, 10 (*p*H 7.8). The "K+-free medium" was similar to the "K+-medium" except that Na^+ replaced all the K^+. The *p*-nitrophenyl phosphate (6.3 mmole/liter) was present as substrate. The ghosts suspensions were incubated at 37°C for 30 minutes, and the reaction was then stopped by cooling and addition of trichloroacetic acid (TCA) (final concentration, 5 percent, weight to volume). Hydrolysis of *p*-nitrophenyl phosphate was estimated by measuring the concentration of nitrophenol (absorption at 410 nm after removal of TCA-insoluble fraction and adjustment of *p*H to 10 with NaOH). The number of ghosts in the final incubation media was adjusted to give a hematocrit reading of about 5 percent in terms of original cells. In all experiments, not less than 60 percent of the ghosts recovered their low permeability to cations. The vertical lines represent the range of three experiments.

Ghosts were prepared (Fig. 1, composition shown in the legend) and, after "sealing" (*6*) and washing, were incubated in isotonic Na^+ or K^+ media with and without $10^{-3}M$ ouabain. The ouabain-sensitive phosphatase activity (*7*) of ghosts rich in K^+ is largely abolished when the ghosts are transferred from a medium containing K^+ to a medium almost free of K^+. Although this result shows that external K^+ is necessary for full activation, it does not rule out the possibility that K^+ is required at both sides of the membrane. To test this point, reconstituted ghosts with no K^+ inside ("Na^+-ghosts") were assayed for ouabain-sensitive phosphatase activity. A large ouabain-sensitive activity can be detected when K^+-free ghosts are suspended in a K^+-containing medium for a length of time that would not lead to a significant intracellular K^+ accumulation (Fig. 1). The lack of effect of intracellular K^+ is further shown by the fact that the activity of the same ghosts remains unchanged after freezing and thawing, although K^+ should now have access to both sides of the membrane because of disruption of the permeability barrier.

Asymmetrical activation of the membrane phosphatase by external K^+, together with the finding (*3*) that the substrate has to be present internally, suggests that the enzyme system responsible for the K^+-activated hydrolysis of *p*-nitrophenyl phosphate has a definite orientation within the membrane. Presumably as a result of this orientation, the substrate can only reach the active site from the internal surface of the cell membrane, whereas the activator can only have access to its specific site from the external surface of the cell membrane. The similitude of these asymmetrical requirements with those of the $(Na^+ + K^+)$-adenosine triphosphatase system (*2*) is consistent with the proposed relation between both enzymic activities.

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References and Notes

1. I. M. Glynn, *Brit. Med. Bull.* **24**, 165 (1968).
2. R. Whittam, *Biochem. J.* **84**, 110 (1962); I. M. Glynn, *J. Physiol.* **160**, 18P (1962).
3. P. J. Garrahan, M. I. Pouchan, A. F. Rega, *J. Physiol.* **202**, 305 (1969).
4. J. F. Hoffman, D. C. Tosteson, R. Whittam, *Nature* **185**, 185 (1960).
5. S. Lepke and H. Passow, *J. Gen. Physiol.* **51**, 365s (1968).
6. "Sealing" means the restoration after hemolysis of the low normal permeability of the red cell membrane to cations after hemolysis. It is accomplished by addition to the hemolyzate of enough concentrated salt solution to restore the tonicity to 315 ideal milliosmols per liter, followed by incubation at 37°C for 40 minutes.
7. Ouabain, having no action in the absence of K^+, selectively abolishes the effect of this cation. For this reason, ouabain-sensitive activity can be taken as synonymous with K^+-dependent activity.
8. Supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas (C.N.I.C.T.), Argentina, and the Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. A.F.R. and P.J.G. are Established Investigators of the C.N.I.C.T.

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Kasugamycin Resistance: 30S Ribosomal Mutation with an Unusual Location on the Escherichia coli Chromosome

Abstract. *A mutation in* Escherichia coli *to resistance to the aminoglycoside antibiotic kasugamycin alters the 30S ribosomal subunit. Though all other known 30S ribosomal mutations are located in a cluster in the streptomycin region, kasugamycin resistance is located at a distance from this region, near the leucine region.*

Mutations to resistance to various antibiotics have proved to be valuable tools for studying the assembly of ribosomes. This communication reports a new locus (*ksg*) affecting the 30*S* ribosomal subunit of *Escherichia coli*, which is unusual in being located on the chromosome at a distance from the known cluster of 30*S* loci in the streptomycin (*str*)-spectinomycin (*spc*) region (*1*, *2*).

Kasugamycin, an antibiotic formed by *Streptomyces kasugiensis*, is widely used