sibility of a different mode of action on these two classes of cells. It is possible, however, that the mode of action of streptomycin on these two classes of cells is identical, but perhaps because of a difference in the permeability of the membranes of the respective cells to streptomycin, there is some interference in the passage of streptomycin to the active site in mammalian cells. Thus a much larger amount of streptomycin would be required to act on these cells. Such a hypothesis may also be evolved to explain the differences in the action of streptomycin and dihydrostreptomycin on mammalian cells. Perhaps these two substances may affect mammalian cells in an identical manner if they can get to the site on which the lethal action occurs, but, because of differences in the permeability of the membranes to these substances, only streptomycin can get to this site under the conditions of the experiment (*9*).

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References and Notes

1. D. P. Metzgar, Jr., and M. Moskowitz, *Exptl. Cell Res.* **30**, 379 (1963).
2. Kindly supplied by Dr. D. Perlman of the Squibb Institute for Medical Research and Dr. W. Sokolski of the Upjohn Company.
3. R. Donovick and G. Rake, *J. Bacteriol.* **53**, 205 (1947).
4. O. E. Landman and W. Burchard, *Proc. Natl. Acad. Sci. U.S.* **48**, 219 (1962).
5. N. Anand and B. Davis, *Nature* **185**, 22 (1960).
6. C. R. Spotts and R. Y. Stanier, *ibid.* **192**, 633 (1961); J. F. Speyer, P. Lengyel, C. Basilio, *Proc. Natl. Acad. Sci. U.S.* **48**, 684 (1962).
7. H. Tzagoloff and W. W. Umbreit, *J. Bacteriol.* **85**, 49 (1963).
8. S. S. Cohen, *J. Biol. Chem.* **168**, 511 (1947).
9. Supported by a grant from the National Science Foundation.

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Gene Action Mechanisms in the Determination of Color and Pattern in the Frog (Rana pipiens)

In urodeles, the cellular basis for pattern differences may be demonstrated by the usual techniques of embryonic transplantation, either of the neural crest, which gives rise to the pigment cells themselves, or the skin prior to its invasion by the migratory crest derivatives (*1*). When these techniques are applied to *Rana pipiens*, the transplants take, but are invariably rejected in the larva prior to the appearance of the definitive adult pattern. Rejection, which may or may not be obvious, is followed by repair from host sources, resulting in a host adult pattern. If pigment repair is incomplete by the time of metamorphosis, permanent pigment-free areas remain in the frog skin, indicating that melanoblasts are incapable of migrating in adult skin. Bovbjerg (*2*) has recently reported rejection of skin transplanted between sibling larvae, in agreement with the findings presented here. Autotransplants are not rejected.

Rejection has been avoided by two means. After exchange of right flank neural crest or dorsolateral skin in Shumway stage 15, the animals were joined in parabiosis (stage 16–17), either by the usual flank methods (*3*), or by the gill primordia. The latter method has proved superior for several reasons. The rich vascular exchange through the gills insures equal growth rates, while flank parabionts frequently become unbalanced, one animal essentially parasitizing the other. These rarely survive as frogs. Gill parabionts survive well and often spontaneously separate following resorption of the gills.

The second method involves exchange of the central belly area along with the underlying mesoderm at stage 17–18. The mesoderm in this region includes those cells destined to give rise to the elements of the blood (*4*). The successful exchange of these *Anlagen* constitutes a sort of permanent transfusion between the animals. The blood-island exchanges have resulted in tolerance in about 30 percent of the operated animals.

Methods of obtaining eggs and operative procedures were those of Hamburger (*3*), with the following exceptions. A 1-mm layer of 5-percent agar was used as the operating substrate, and the animals were kept in full strength Holtfreter's solution until corresponding hatching stages. Larvae were reared on parboiled spinach and the frogs on crickets.

Two well-known pattern mutants occur at low frequency in the Minnesota populations (*5*). The first variety, designated "burnsi," proved to be a single, incompletely dominant gene acting to inhibit the normal spotting pattern (*6*). The second mutant known as "kandiyohi" also is a single, dominant gene introducing mottled marks into the nonspotted areas of the skin (*7*). The two loci express their effects independently. Four classes may be obtained in equal frequency by crossing a burnsi heterozygote with a kandiyohi heterozygote (*5*). These are (i) the normal spotted frog, (ii) the burnsi frog with reduced spotting, (iii) the kandiyohi frog with normal spotting and mottled background skin, and (iv) the double mutant, kandiyohi-burnsi, a mottled frog with reduced spotting. Random exchanges of grafts between embryos from such a cross result in 75-percent combinations between animals of different genotypes, the remaining 25 percent being combinations between animals of the same genotype.

Exchanges of neural crest demonstrate the specificity of both the burnsi and kandiyohi loci as well as the double mutant. Dorsolateral skin exchanges come through strictly as host, as do simple blood-island exchanges and parabioses, the latter ruling out hormonal factors as primary genetic differences. In addition to the pattern

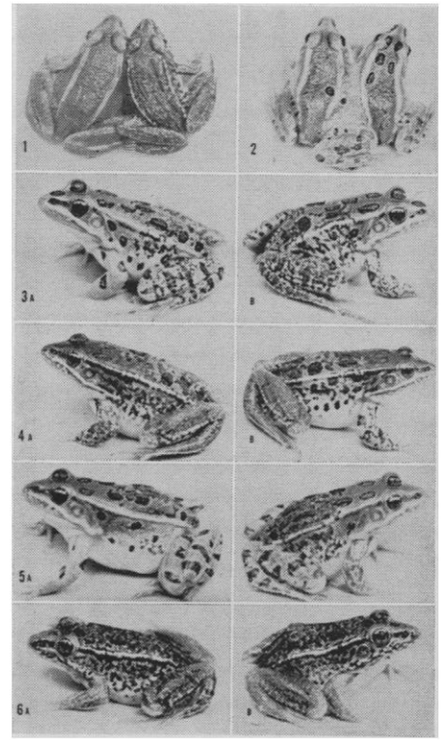

Fig. 1. The mutant types, burnsi (left) and kandiyohi (right), in gill parabiosis. Fig. 2. Right crest exchange followed by flank parabiosis between burnsi (left) and normal (right). Fig. 3. Left (*A*) and right (*B*) views, respectively, of a normal host with kandiyohi right crest. Fig. 4. Left (*A*) and right (*B*) views of kandiyohi host with normal right crest. Fig. 5. Left (*A*) and right (*B*) views of normal host with double mutant (kandiyohi-burnsi) right crest. Fig. 6. Left (*A*) and right (*B*) views of kandiyohi host with double mutant right crest. See the text for the explanation of tolerance induction.

mutants, skin color was unexpectedly found to be under the control of the crest derivatives. The inheritance of skin color is still unclear but, judging from the wide spectrum of hues and intensities, the genetic mechanism is probably complex.

Figures 1–6 illustrate representative effects observed. Figure 1 illustrates the two mutant types, burnsi (left) and kandiyohi (right), joined in gill parabiosis. Figure 2 is a flank parabiosis of a normal (right) and burnsi (left), in which the right-hand neural crests were exchanged. Note the appearance of normal spots on the right side of the burnsi animal and their absence in the normal. In this case considerable lateral mixing probably occurred in the flank regions supplying the hind legs. Not clear from the photo is a pronounced color difference. The burnsi is brown while the normal is green except in the region where the spotting is reversed. Here the burnsi is green while the normal is brown. The color differences blend laterally but not longitudinally with the host colors, again indicating lateral mixing following closure of the neural folds. Not evident in dorsal view, the two inner forelegs are spotted, the outer spotless. The remaining pictures are left, or host (*A* in Figs. 3–6), and right, or donor (*B* in Figs. 3–6), views of the same animal which has received right flank crest from the genotype indicated. Figure 3 shows a normal host which received crest and blood island from a kandiyohi donor. Notice the kandiyohi mottlings are limited largely to the right or donor side. Figure 4 shows the reciprocal animal, a kandiyohi frog which received crest and blood island from the normal in Fig. 3. Compare especially the flank and forelimbs. Figure 5 is a normal host which received right crest and blood island from a double mutant donor (kandiyohi-burnsi). In this case very little mixing occurred as the effect is largely unilateral. Figure 6 shows a kandiyohi gill parabiont which had received double mutant right crest. The animals spontaneously separated after metamorphosis. The effect here is largely limited to the hind limbs, which are spotted in the host view and spotless in the donor view. The reciprocal animal, not pictured, shows the reciprocal effects. Notice in the left view the absence of a well-defined tympanic membrane. This is characteristic of the separated gill parabionts and is the only visible defect marking the region of original attachment.

I conclude from these observations that the burnsi and kandiyohi loci, as well as those genes responsible for color differences, all act through modifications in the pigment-forming elements themselves, rather than in the skin substrate through which these cells aggregate and differentiate to determine the adult phenotype (*8*).

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References and Notes

1. H. E. Lehmann and L. M. Youngs, in *Pigment Cell Biology*, M. Gordon, Ed. (Academic Press, New York, 1959).
2. A. Bovbjerg, *Am. Zoologist* **2**, 508 (1962).
3. V. Hamburger, *A Manual of Experimental Embryology* (Univ. of Chicago Press, Chicago, 1960).
4. H. Federici, *Arch. Biol. Liege* **36**, 466 (1926).
5. S. C. Anderson and E. P. Volpe, *Science* **127**, 1048 (1958).
6. J. A. Moore, *Genetics* **27**, 408 (1942).
7. E. P. Volpe, *Systematic Zool.* **4**, 75 (1955).
8. Supported by grants from the National Institutes of Health (H-5840) and the National Science Foundation (B-13342). I express my appreciation to T. C. Mayer (Ryder College), who introduced me to the techniques of embryonic transplantation.

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Sex Chromatin Patterns and the Lyon Hypothesis

Abstract. *Polyploid human cells in culture often have paired sex chromatin masses. They are neither chance juxtapositions of randomly distributed X chromosomes nor manifestations of a propensity for pairing. The pairs are characteristic of the first polyploid interphase. They are thought to reflect the persistent differentiation of X chromosomes, as proposed in the Lyon hypothesis.*

Many investigators have noticed that (presumably) tetraploid cells arising from diploid, diplo-X human cells in culture have two sex chromatin masses (SCM's) instead of the single one found in the diploid state. Some published photographs of such cells (*1*) have the feature in common of depicting the two SCM's close together. These pairwise arrangements are found whether the SCM's are on the lateral margin of the flattened nuclei or not. If one considers, for the sake of simplicity, only those nuclei in which both SCM's are on the lateral margin, the nonrandom nature of the paired arrangements can easily be demonstrated. Each such nucleus can be characterized by a ratio relating the shorter segment of the perimeter separating the two SCM's (= *D*) to the total perimeter (= *P*). If the SCM's are distributed about the perimeter at random with regard to one another, then all values of *D/P* are equally likely. I determined *D/P* as follows: cultures of diploid, diplo-X human cells derived from the skin were grown on cover slips (*2*). The cells were fixed in absolute ethanol (3 parts) and glacial acetic acid (1 part), treated with 1*N* HCl (60°C for 11 to 12 minutes), and stained with the Feulgen reaction. The cover slips were scanned systematically and nuclei having two peripheral SCM's in reasonably sharp, simultaneous focus were photographed as encountered. The negative images were projected and traced. From the tracings, *D* and *P* were determined with a map measurer.

The frequency distribution of *D/P* is shown in Fig. 1 (solid bars). It is not a random distribution ($\chi^2 = 180$; $P \ll .001$) but exhibits a marked predominance of nuclei with closely associated SCM's. The same sort of analysis was made with the nuclei of cultured triplo-X cells (*3*), which also have two SCM's but in the diploid state. In this case, the frequency distribution of *D/P* (Fig. 1, dotted bars) does not significantly differ from randomness ($\chi^2 = 4.40$; $0.80 < P < 0.90$). It shows that there is no *general* tendency for two SCM's of the same nucleus to be associated and that pairwise arrangements

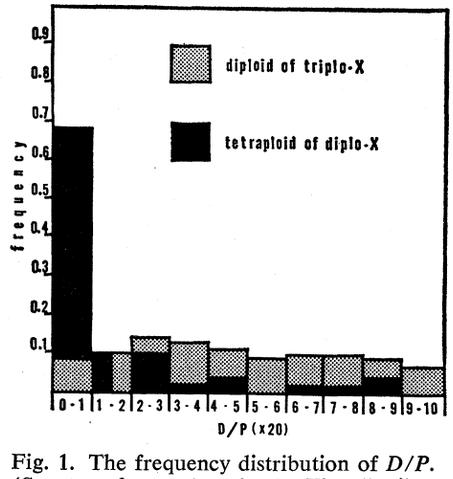

Fig. 1. The frequency distribution of *D/P*. (See text for explanation.) The distributions are based on 100 diploid nuclei of triplo-X origin and 50 tetraploid nuclei of diplo-X origin.