horizontal motions and the horizontal scales of such circulations are expected to be very much larger than 100 m.
20. M. D. Palmer, *J. Geophys. Res.* **78**, 3585 (1973); U. Lemmin, J. T. Scott, U. H. Czapski, *ibid.* **79**, 3442 (1974).
21. Technically, the spectrum of a passive scalar, like chlorophyll concentration, may differ in shape from the spectrum of velocity fluctuations. However, this difference can only occur at very high and very low wave numbers. At very high wave numbers, corresponding to scales of the order of a centimeter and smaller, molecular processes are important, and the molecular diffusivity of a phytoplankton cell differs from the molecular kinematic viscosity of water. These scales are far below our spatial resolution ($\simeq$ 5 to 10 m). At very low wave numbers—scales approaching the size of the sources of variance—the spectral shapes should diverge if the fluctuations in velocity are caused by agents with very different size scales than those which cause the fluctuations in chlorophyll concentration. Since our experiment samples the low wave number region, we attribute the observed divergence of the spectra to different source agents; that is, the chlorophyll variation at large scales cannot be due to turbulence alone, but must be attributed to biological processes which act over large scales as well. See G. K. Batchelor, *J. Fluid Mech.* **5**, 113 (1959).
22. C. R. Goldman, G. Moshiri, E. de Amezaga, in *Water Pollution Control in Cold Climates*, R. S. Murphy and D. Nyquist, Eds. (No. 5501-0208, Government Printing Office, Washington, D.C., 1972), p. 1.
23. P. J. Richerson, B. J. Dozier, B. T. Maeda, *Verh. Int. Ver. Limnol.*, in press.
24. H. Tennekes and J. L. Lumley, *A First Course in Turbulence* (MIT Press, Cambridge, Mass., 1972).
25. J. F. Haney, thesis, University of Toronto, Ontario (1970); P. J. Richerson, in *Proceedings of Seminar on Eutrophication and Biostimulation*, R. L. Brown and M. G. Tunzi, Eds. (California Department of Water Resources, Sacramento, 1972), p. 133.
26. E. A. Stull, E. de Amezaga, C. R. Goldman, *Verh. Int. Ver. Limnol.* **18**, 1776 (1973); T. R. Parsons and M. Takahashi, *Limnol. Oceanogr.* **18**, 511 (1973); R. E. Hecky and P. Kilham, *ibid.* **19**, 361 (1974); T. R. Parsons and M. Takahashi, *ibid.*, p. 366.
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The Possible Role of Histones in the Mechanism of Chromosomal G Banding

Abstract. *Cytochemical data are presented to show that the histone fractions f1 and f2a are involved in the induction of chromosomal G bands, whereas the f2b and f3 fractions are not involved. Removal of the f1 and f2a fractions probably occurs during fixation and is necessary for the induction of G bands.*

During recent years a number of procedures have been devised to induce cross-bands, now known as G bands, of metaphase chromosomes. These procedures include treating fixed metaphase chromosomes with proteolytic enzymes, alkylating agents, protein denaturants, phosphate buffer at high temperature, and many others. The G bands are extremely useful in identification of individual chromosomes and their arrangements, but the mechanism by which G bands are induced remains controversial. It has been suggested that alterations of protein-DNA relationships allow the Giemsa mixture to differentially stain specific regions on the chromosomes (*1–3*). Whether histones or acidic proteins (or both) are involved remains unclear (*1, 2*). We present cytochemical data to show that the histone fractions f1 and f2a are at least partially responsible for the induction of G bands, whereas the f2b and f3 fractions are not involved.

The Chinese hamster fibroblast line Don and the cells of the cactus mouse, *Peromyscus eremicus*, were grown as monolayers in McCoy's 5a medium with 20 percent fetal calf serum. Cells were fixed in Carnoy's mixture (methanol and acetic acid, 3 : 1), placed on slides by the air-dried technique, and G bands induced with trypsin (*4*). After treatment with trypsin a few slides were stained with Giemsa to ensure that G band induction was properly done. The various histone fractions (Sigma) and cytochrome c (Sigma) were dissolved in 5 m*M* $MgCl_2$ and 50 m*M* tris-HCl, *p*H 7.5, at a concentration of 0.1 or 1 mg/ml. Calf thymus DNA, either native or heat denatured, was dissolved in the above buffer at a concentration of 1 mg/ml. Half of a trypsin G banded slide was treated with the solution containing one of the above proteins or DNA, while the other half was treated with only the buffer. The slides were incubated at room temperature for either 5 or 20 minutes, and treated by one of the following methods prior to staining with Giemsa: (i) rinsed, (ii) trypsinized, or (iii) fixed in methanol and acetic acid (3 : 1) or 0.2*N* HCl for 30 minutes.

The responses of all chromosomes in each experiment were the same, but for the sake of simplicity, we chose to use only the Chinese hamster chromosome 2 to illustrate the staining behavior. In all slides, control sections showed typical G banding (Fig. 1A). When the trypsin-treated section was incubated with the lysine-rich histone fraction, f1, at a concentration of 0.1 mg/ml, G bands were preserved when the preparation was stained with Giemsa (Fig. 1B). However, when similar slides were treated with f1 at 1 mg/ml, not only the G bands disappeared, but the chromosomes did not take up stain at all, giving a ghost chromosome appearance (Fig. 1C). The slightly lysine-rich fraction, f2a, showed similar behavior, that is, G bands were not blocked at a concentration of 0.1 mg/ml (Fig. 1D), but were completely obliterated at 1 mg/ml (Fig. 1E). Unlike f1, the chromosomes incubated with f2a did not give a ghostlike appearance although they were not intensively stained. No demonstrable reaction was noticed when the slightly lysine-rich fraction, f2b, and the arginine-rich fraction, f3, were used for incubation at either of the concentrations and durations used in our experiments (Fig. 1, F and G, respectively). As controls we used the basic protein cytochrome c (Fig. 1H) and the neutral protein egg albumin (not shown). Neither showed any effect on the appearance of G bands. Denatured and native DNA were also ineffective in blocking the G banding.

Our data suggested that the f1 and f2a histone fractions may be involved in the production of G banding of the chromosomes, but each appeared to have a different mode of operation. The f1 histone completely blocks the staining of the chromosomes, whereas f2a does not. In another set of experiments, we used air-dried slides without first subjecting them to trypsin treatment, and treated them with either f1 or f2a, and then stained with Giemsa. The f1 histone here inhibited Giemsa staining

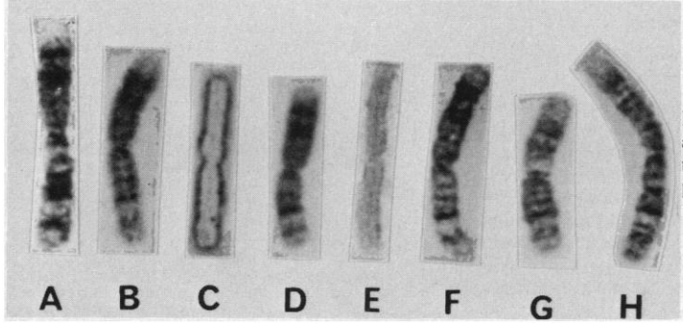

Fig. 1. G banding pattern of chromosome 2 of Chinese hamster fibroblast after treatment with trypsin followed by the protein at the noted concentrations for 5 minutes and finally stained with Giemsa. (A) Control (no protein); (B) f1, 0.1 mg/ml; (C) f1, 1 mg/ml, (D) f2a, 0.1 mg/ml; (E) f2a, 1 mg/ml; (F) f2b, 1 mg/ml; (G) f3, 1 mg/ml; (H) cytochrome c, 1 mg/ml.

similar to the one shown in Fig. 1C, while the f2a had no demonstrable effect on the staining of the chromosomes. The results suggest that the removal of the f1 histone is necessary prior to staining with Giemsa. To substantiate this interpretation, we treated the slides with trypsin (induction of G banding), added f1 and f2a histones (blocking of G banding), treated the cells with trypsin again, and stained with Giemsa. After the second trypsin treatment the chromosomes exposed to the f1 were again banded. However, exposure to the f2a and subsequent trypsin treatment induced no bands, even when the duration of the trypsin treatment was increased.

Another approach was to induce bands on the chromosomes with trypsin, block with either the f1 or f2a fraction, fix again in Carnoy's mixture or 0.2*N* HCl, and stain. This procedure was used to study the effect of fixation on removal of the histones. G bands reappeared. Therefore our data indicated that fixation in Carnoy's mixture or 0.2*N* HCl removed the histone fractions f1 and f2a from the chromosomes, thus allowing for the reappearance of the bands.

It has been reported that fixation in a mixture of ethanol and acetic acid (3 : 1) removed 7 to 8 percent of the total histone from calf thymus chromatin (*5*) and fixation in Carnoy's mixture removed 8 to 20 percent of the histones from nuclei (*2*). These results have been used to support the hypothesis that the histones are not involved in the mechanism of banding. Our data suggest that the histones play an important role in the mechanism of banding. The removal of the f1 and f2a fractions most likely occurs during fixation and appears to be a prerequisite for G banding. Since the Giemsa stain does not seem to interact with the f1 histone fraction, it may be necessary to remove the f1 fraction for staining chromosomes with Giemsa.

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References and Notes

1. C. L. Y. Lee, J. P. Welch, S. H. S. Lee, *Nat. New Biol.* **241**, 142 (1973).
2. D. E. Comings and E. Avelino, *Exp. Cell Res.* **86**, 202 (1974).
3. W. Vogel, J. Faust, M. Schmid, J. W. Siebers, *Humangenetik* **21**, 227 (1974).
4. S. Pathak, T. C. Hsu, L. Shirley, J. D. Helm, *Chromosoma* **42**, 215 (1973).
5. C. Dick and E. W. Johns, *Exp. Cell Res.* **51**, 626 (1968).
6. Supported by NSF research grant GB-41296 and NIH research grant 1 T22 GM-00143. We thank Drs. F. E. Arrighi and E. Stubblefield for critical reading of the manuscript.

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Goldfish Abducens Motoneurons: Physiological and Anatomical Specialization

Abstract. *During natural movements, the motoneurons innervating a single muscle have different patterns of activity that are correlated with differences in synaptic input. The caudal abducens motoneurons fire phasically in synchronous bursts before rapid posterior eye movements; the rostral abducens motoneurons fire only tonically when the eye is fixed or moving slowly. This physiological difference is not related to motoneuron size. In this respect the abducens motoneurons violate the "size principle" that has been advanced for spinal motoneurons. The difference is probably related to the present finding that the caudal but not the rostral cells receive numerous electrical synapses that are known to have a role in synchronizing phasic activity.*

A vertebrate muscle is a collection of motor units, each consisting of a group of muscle fibers innervated by a single motoneuron. Fibers within a particular motor unit are quite homogeneous, but the motor units within a single muscle often exhibit marked physiological, biochemical, and morphological specialization. A rough classification of fibers into "fast" (or "phasic") and "slow" (or "tonic") types is generally recognized (*1*). It has been suggested that the nervous system may have mechanisms for activating different types of motor units in various combinations and patterns. For example, ballistic movements might be initiated by phasic motor units while other types of movements might begin with mobilization of slow motor units capable of long-lasting, tonic activity (*2*).

In support of this idea, Burke and his colleagues (*3*) have demonstrated qualitative physiological differences in the synaptic input to the motoneurons that supply fast and slow motor units in a single muscle. However, it has not yet been demonstrated that the different types of motoneurons supplying a single muscle actually show different activation patterns in naturally occurring movements, nor has it been shown anatomically that different types of motoneurons supplying a single muscle have qualitatively different synaptic inputs. This has been particularly difficult, since in most cases the neurons supplying the different types of motor units are intermingled and are not anatomically distinguishable. We describe here a part of the goldfish oculomotor system in which there is an extremely good correlation between naturally occurring fast and slow movements, anatomical specialization of fibers within a single muscle, and the physiological specialization of its motoneurons. An additional unique feature of the system is that the two physiological classes of motoneurons are grouped in distinct subnuclei. This has permitted us to demonstrate that the two groups of motoneurons differ in their synaptic inputs.

The posterior rectus muscle moves the fish's eye from anterior to posterior in the horizontal plane. Under different conditions, movement can occur as a rapid "saccade" at velocities up to 400° per second or as a slow drift. The muscle also acts in fixing the eye in various positions in the anterior-posterior axis (*4*). The goldfish posterior rectus is about 17 mm long and is composed of about 1100 fibers of two distinct types that are sharply segregated within the muscle (*5*) (Fig. 1B). The large fibers (Fig. 1A) are 8 to 32 μm in diameter, contain few mitochondria, and have no capillary supply. The small fibers (Fig. 1C) are 5 to 14 μm in diameter and, in contrast to the large fibers, are richly supplied with mitochondria and capillaries. Although no physiological or histochemical studies have been performed on this muscle, there are good reasons to believe from studies of other muscles that the large fibers are anaerobic and fast contracting and fatiguing, while the small fibers are aerobic and slowly contracting and fatiguing (*1*).

The motoneurons innervating the posterior rectus lie in two distinct cell groups in the medulla that are separated by about 200 μm in the rostro-caudal axis (Fig. 1F). Each group contains about 50 cells that, when measured in light microscope sections 50 μm thick, range from 11 to 30 μm in diameter. The distribution of cell sizes is the same for the two groups ($P > .89$). Axons from large and small neurons (arrows, Fig. 1F) in each group can be traced directly into rootlets that fuse to form the abducens nerve. The nerve, which in fish innervates only the posterior rectus (*6*), contains about 100 axons that range from 1 to 20 μm in diameter. The good correspondence between the number of axons in the nerve and the number of cell bodies suggests that most of the cells in each group are motoneurons. Furthermore, they must be alpha and not gamma motoneurons since fish do not have muscle spindles (*7*). All these considerations lead to the conclusion that the functional differences we found between the two cell groups cannot be attributed to differences in cell size.

We examined the functions of the two