protein fraction from rat skeletal myofibrils exhibited very low activity.

These results demonstrate the ubiquitous presence of small amounts of an ankyrin-like protein distinct from myosin or filamin which is associated primarily with membrane components. Ankyrin links band 3 to the erythrocyte cytoskeleton[4], and may have a related function in specialised regions of the plasma membrane in other cells. Spectrin was not detected in cultured cells by radioimmunoassay[14], but this negative result could be explained by poor cross-reactivity of the antibody or by insufficient sensitivity of the assay. It is conceivable that proteins analogous to ankyrin, spectrin and band 3 could be present together in non-erythrocyte cells, and could be involved in processes requiring direct contact between integral membrane proteins and structural proteins on the inner surface of the membrane.

The technical assistance of Peter J. Stenbuck and growth of cultured cells by Lydia J. Hernaez are acknowledged.

Received 16 July; accepted 20 August 1979.

1. Bennett, V. & Stenbuck, P. J. *J. biol. Chem.* **254,** 2533–2541 (1979).
2. Luna, E. J., Kidd, G. H. & Branton, D. *J. biol. Chem.* **254,** 2526–2532 (1979).
3. Yu, J. & Goodman, S. *Proc. natn. Acad. Sci. U.S.A.* **76,** 2340–2344 (1979).
4. Bennett, V. & Stenbuck, P. J. *Nature* **280,** 468–413 (1979).
5. Bennett, V. *J. biol. Chem.* **253,** 2292–2299 (1978).
6. Hunter, W. M. & Greenwood, F. C. *Nature* **194,** 495 (1962).
7. Goding, J. W. *J. immun. Meth.* **18,** 183–192 (1978).
8. Bennett, V. & Branton, D. *J. biol. Chem.* **252,** 2753–2763 (1977).
9. Steck, T. L. *J. Cell Biol.* **62,** 1–19 (1974).
10. Boyam, A. *Scand. J. clin. Lab. Invest.* **21,** Suppl. 97, 77–89 (1968).
11. Perper, R. J., Zee, T. W. & Mickelson, M. M. *J. Lab. clin. Med.* **72,** 842–849 (1968).
12. Rodbell, M. *J. biol. Chem.* **239,** 375–380 (1964).
13. Fairbanks, G., Steck, T. L. & Wallach, D. F. H. *Biochemistry* **10,** 2606–2617 (1971).
14. Hiller, G. & Weber, K. *Nature* **266,** 181–183 (1977).

A mutation in *Caenorhabditis elegans* that increases recombination frequency more than threefold

A. M. Rose & D. L. Baillie

Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada

In higher organisms the rate of recombination between genetic loci is presumably responsive to selective pressure. Recently, selective pressures[1] and mutational events[2] that influence recombination have been reviewed. Mutational sites and chromosomal rearrangements that enhance or suppress recombination frequency in specific regions are known, but general mechanisms that enhance recombination have not yet been discovered. We describe here the isolation and characterisation of a strain of the hermaphroditic nematode, *Caenorhabditis elegans*, that has a recombination frequency at least threefold higher than that found in the wild type[3,4]. In this strain, *rec-1*, the number of reciprocal recombination events between linked loci is increased. This is true for all pairs of linked loci studied so far. The high recombination strain behaves as if it carries a classical, recessive mutation, although a second mutation exists which can alter the recessive behaviour of *rec-1*.

We established a strain heterozygous for mutations in closely linked loci on chromosome I. This strain, *dpy-5 unc-15*+/++*unc-13*, has a wild-type appearance. When allowed to self-cross it segregates dumpy paralysed (*dpy-5 unc-15/dpy-5 unc-15*), uncoordinated (*unc-13/unc-13*) and wild-type appearing (*dpy-5 unc-15*+/++*unc-13*) progeny. *dpy-5 unc-15/dpy-5 unc-15* are short, fat worms that cannot move, and *unc-13/unc-13* have a characteristic contorted appearance and movement. In addition to these phenotypes, recombinant dumpy (*dpy-5*+/*dpy-5 unc-15*) and paralysed (+*unc*-15/*dpy-5 unc-15*) progeny will occur. By selecting the hermaphrodites with wild-type appearance, this strain was maintained for several generations, and eventually scored for recombination between the *dpy-5* and *unc-15* loci. The frequency of recombination in this region was known from previous experiments[4] to be approximately 2% at 20 °C when all the progeny of a single parent were scored. In the heterozygous strain that we were maintaining, the recombination frequency between *dpy-5* and *unc-15* was approximately 7%. We hypothesised that our *unc-13/unc-13* strain, used to make the heterozygous strain, carried a factor responsible for this increase in recombination frequency. To demonstrate the existence of this factor, we out-crossed our uncoordinated strain to wild type and generated *unc-13*/+ males. These males were crossed to *dpy-5 unc-15/dpy-5 unc-15*. On self-crossing, half the F_1 heterozygotes from this cross showed a high frequency of recombination among their progeny, while the other half had a normal recombination frequency. Successive generations of the high recombination individuals were measured for recombination frequency until a homozygous strain of high recombination hermaphrodites (XX) was established. This strain was treated at 26 °C for 30 h to produce males (X0) by X-chromosome nondisjunction. When these males were crossed to *dpy-5 unc-15/dpy-5 unc-15*, all the F_1 heterozygotes segregated a high number of recombinant individuals. After elimination of *dpy-5*, *unc-15* and *unc-13* mutant alleles by recombination, we established a male strain homozygous for *rec-1*, but having no visible mutant phenotype.

Table 1 The effect of *rec-1* on recombination frequency (%)

	Parental genotypes		
ab	*ab*/++	*ab*/++; *rec-1*/+	*ab*/++; *rec-1*/*rec-1*
dpy-5 unc-13 (I)	1.51±0.3*	1.30±0.3	12.41±1.6
dpy-14 unc-56 (I)	†	0.61±0.2	4.14±1.5
unc-22 unc-43 (IV)	1.90±0.6	1.55±0.4	3.95±1.4
dpy-11 unc-42 (V)	2.21±0.3	2.60±0.7	7.92±1.8

* ±95% confidence intervals.
† Not determined.

Table 2 The influence of *s155* on the expression of *rec-1*

	Parental genotypes	
ab	*ab*/++; *s155*/+	*ab*/++; *s155*/+ *rec-1*/+
	Recombination (%)	
dpy-5 unc-13 (I)	1.15±0.3	7.34±0.9
dpy-5 unc-15 (I)	1.80±0.4	9.11±0.9
dpy-5 dpy-14 (I)	1.62±0.6	5.50±0.9

Males from this strain were crossed to *dpy-5 unc-13/dpy-5 unc-13* individuals. Unlike before, all the F_1 heterozygotes produced a normal number of recombinants among their progeny. In the F_2, two types of heterozygote occurred—those which gave a normal frequency of recombinants and those which gave a high frequency of recombinants. Approximately one-quarter of the F_2 heterozygotes had high recombination frequencies and produced true-breeding high recombination progeny. In a parallel control experiment, *dpy-5 unc-13/dpy-5 unc-13* were crossed to +/+ males and scored for recombination frequency in the F_1, F_2 and F_3. In this case the recombination frequency remained approximately 2% in every generation. From these studies, we concluded that the *rec-1* strain has a segregating factor which assorts independently of chromosome I. This factor is responsible for at least a threefold elevation in recombination frequency.

To investigate the generality of this increased recombination phenomenon, *rec-1/rec-1* males were crossed to other pairs of linked loci on chromosomes I, IV and V. The results showed that *rec-1* acts recessively to increase recombination frequency between two pairs of linked loci on chromosome I (Table 1). On

0028–0836/79/420599—02$01.00

chromosomes IV and V, recombination frequency in the *unc-22 unc-43* and the *dpy-11 unc-42* regions, respectively, was measured. Here also, an increase in recombination frequency was observed (Table 1). We interpret these results as evidence that *rec-1* is a general enhancer of recombination frequency in *C. elegans*.

With certain pairs of linked loci, a high recombination frequency was observed in the F_1 generation. For example, when *rec-1* males were crossed to *dpy-5 unc-15/dpy-5 unc-15* or *dpy-5 dpy-14/dpy-5 dpy-14*, the F_1 heterozygotes had a higher recombination frequency than wild type. From these results we hypothesised that *dpy-5 unc-15/++* and *dpy-5 dpy-14/++* individuals carry a site, *s155*, responsible for the dominant behaviour of the high recombination factor.

A strain, homozygous for *dpy-5 unc-13* and *s155* was constructed. If *s155* is, in fact, responsible for conferring dominant behaviour on *rec-1*, then *s155*-carrying *dpy-5 unc-13/dpy-5 unc-13* individuals should behave differently from *dpy-5 unc-13/dpy-5 unc-13* individuals when crossed to *rec-1*. Table 2 shows that when pairs of loci on chromosome I are tested in the presence of *s155*, *rec-1* acts dominantly. This provides evidence that a second mutation can modify the recessive nature of *rec-1*. Experiments are in progress to test whether *s155* can confer dominance on regions on other chromosomes.

We believe this to be the first demonstration of a general enhancer of recombination frequency in a higher eukaryote. The existence of a gene that can alter recombination and that is susceptible to mutation provides a mechanism whereby recombination frequency can be selected. It may be possible that, in addition to this allele of *rec-1*, other alleles which decrease recombination frequency can be recovered. If so, perhaps different levels of recombination frequency are selected in exclusively self-crossing or exclusively out-crossing populations. Furthermore, our *rec-1* strain may have an elevated spontaneous mutation rate. Three new visible phenotypes independent of *rec-1* have been observed in this strain. Thus, it is possible that the mechanism by which *rec-1* acts also alters the mutation frequency. This and other questions regarding the mode of action of *rec-1* are being investigated.

We thank Clara Salamanca for technical assistance, Raja Rosenbluth and Drs D. G. Holm, L. M. Dill, A. T. Beckenbach, M. J. Smith and J. M. Webster for discussion, our reviewer for his comments on the manuscript, and the NSERC and MDA of Canada for financial support.

Received 15 June; accepted 22 August 1979.

1. Maynard-Smith, J. *The Evolution of Sex* (Cambridge University Press, 1978).
2. Catcheside, D. G. *The Genetics of Recombination* (University Park Press, Baltimore, 1977).
3. Brenner, S. *Genetics* **77**, 71–94 (1974).
4. Rose, A. M. & Baillie, D. L. *Genetics* (in the press).

Correlation between fragmented immunoglobulin genes and heavy chain deletion mutants

Blas Frangione* & Edward C. Franklin

Irvington House Institute, Departments of Pathology* and Medicine, New York University Medical Center, 550 First Avenue, New York, New York, 10016

It is generally accepted that the variable (V) and constant (C) regions of immunoglobulin (Ig) chains are under separate genetic control[1]. The notion that the different domains and interdomain regions are also under the control of independent genetic units was initially based on the clearcut results obtained by studying the primary structure of deletion mutants[2–9] and received definitive support from direct analysis of cloned heavy (H) and light (L) chain genes[10–13]. Here we present additional studies carried out on two selected γ3 deletion mutants which indicate that the genetic control of human H chains may be even more complex than previously believed.

L and H chains of Ig molecules consist of two functionally differentiated segments: the amino-terminal V region which contains the antigen binding sites, and the carboxy-terminal C region[14]. Sequence and crystallographic studies have shown that the C region of the IgG H chain is composed of three domains: CH1, CH2 and CH3. The striking structural homology among the C region domains, each of which is composed of about 110 residues folded into an almost identical tertiary structure, and the lesser homology to the V regions suggest a common evolutionary origin by a series of gene duplications. Each domain has a well defined function. The CH1 domain contains the attachment point for the L chain and pairs with the CL domain; together with paired VH and VL domains containing the antigen binding sites they form the Fab fragments of the IgG molecules. The CH2 domains, which act in complement fixation, and the CH3 domains, which are involved in cell surface interactions together form the Fc fragment. In the middle of the H chains of all subclasses of IgG, there is a region of 15–62 residues of unknown origin and function called the hinge region or Fh fragment, which separates the Fab and Fc fragments[15,16]. Thus, the γ H chain is composed of five discrete structural and functional units.

Heavy chain disease (HCD) protein SPA is an 80,000-molecular weight (MW) dimeric γ3 human H chain mutant with a blocked amino terminus. The amino-terminal sequence after unblocking is: <Glu-Glu-Glu-Val-Arg-Glu-Ser-Glu-Leu-Lys-Thr-Pro-Leu-Gly-Asp-Thr-Thr-(His)-Thr-Cys-Pro-(Pro)-Cys-Pro. Comparison with the prototype VH amino-terminal sequences shows that although the first seven residues bear some resemblance to the normal amino terminus they do not correspond to any known VH subgroup or CH1 sequence[25]. Position 8 is the beginning of the normal γ3 hinge region[16]. On the basis of its amino acid analysis and high cysteine content it seems

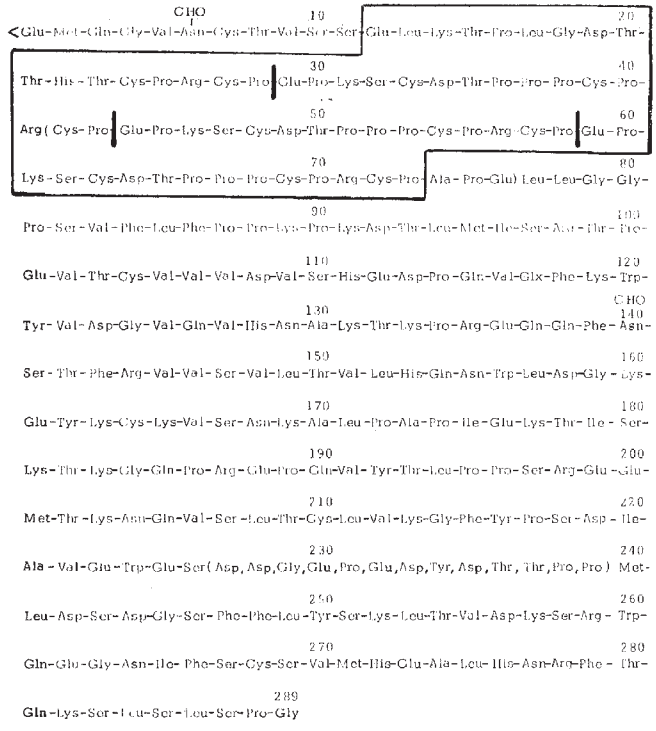

Fig. 1 The amino acid sequence of human γ3 heavy chain deletion mutant WIS. Position 1 to 7 is an unusual V sequence; position 8 to 11 corresponds to VC or junction sequence; position 12 is the beginning of the hinge region of normal γ3 heavy chain which is 62 amino acid residues long (inside box) and four homologous subunits indicated by vertical bars. The first is a 17-residue segment followed by a 15-residue segment which is identically and consecutively repeated three times. CHO: carbohydrate; <Glu: 5-pyrrolidone-2-carboxylic acid.

0028-0836/79/420600—03$01.00