ute, the calories expended for temperature regulation are easily replaced if the flowers contain their full complement of nectar.

Many bees and other insects, as well as hummingbirds, take nectar from *E. angustifolium* and thereby reduce the amounts of sugar that are available in the flowers at any one time. For example, the unscreened flowers (examined an hour after the screened) contained a mean of only 0.086 mg of sugar (range, 0.00 to 0.17 mg; $N=51$). However, despite the relatively low sugar content of these flowers, *B. vagans* should make an energetic profit from them at low T_A if each bee extracts nectar from more than 1.7 flowers per minute. The above approximations suggest that the energetic cost of temperature regulation is justified in *B. vagans* foraging from *E. angustifolium* at relatively low T_A, especially if the bees can harvest nectar at times when other insects are excluded from the flowers.

The bumblebees are probably seldom required to actively dissipate heat from the thorax while foraging; under maximum thermal conditions observed in the field (noon sunshine at 31°C), T_{Th} tends to be no higher than 40°C (Fig. 1). Since the abdomen is often heated by solar radiation and approaches T_{Th}, and since heat loss by forced convection from the abdomen is greatly reduced when the bees are walking on flowers, the abdomen obviously could not act as an efficient heat dissipater for excess heat from the thorax during foraging, as in the sphinx moth *Manduca sexta* [see (*19*)].

In hovering sphinx moths, heat is produced continuously at a high rate. In *M. sexta* during free and continuous flight at $T_A > 23$°C, T_{Th} is regulated at the upper tolerable temperature level (40° to 43°C) by shunting the excess heat (via the blood) from the thorax (*19*). However, in foraging bumblebees that land on flowers, the durations of obligatory heat production are brief; the bees are in continuous flight between individual flowers for only 1 to 3 seconds. Heat production is optimal during the stationary periods, and *B. vagans* regulates T_{Th} near the lower limit of T_{Th} [see (*9*)], at which temperature flight between the scattered and calorically rewarding flowers of *E. angustifolium* is possible. However, bumblebees on dense inflorescences often have a lower T_{Th} when the caloric contents of the flowers are low, whereas under other conditions T_{Th} can be relatively high when bees are foraging at low T_A (*20*).

Temperature regulation in bumblebees has been treated separately in relation to food resources, foraging strategies, and floral morphology (*20*).

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8. The T_{Th} of seven *B. ternarius* returning to their nest at a T_A of 11° to 15°C ranged from 32.6° to 36.0°C (arithmetic mean = 34.7°C) (*B. ternarius* is nearly the same size as *B. vagans*). The T_{Th} of five bees leaving their nest during the same time ranged from 33.8° to 36.0°C (mean = 34.5°C). The bees were caught by net.
9. Bumblebees can fly clumsily with a T_{Th} of 29°C, but *B. terricola* returning to the nest at a T_A of 31°C had a T_{Th} of 42.5° to 43.3°C.
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12. Bumblebees that were "cold" and could walk only slowly and deliberately sometimes warmed up rapidly when touched. During warm-up the wings were folded dorsally, wing movement was not apparent, and buzzing could not be heard. However, the frequency and amplitude of abdominal respiratory movements increased rapidly throughout warm-up until flight was initiated. (In Lepidoptera, which are neurogenic rather than myogenic, the wings beat during warm-up, and ventilation of the thoracic musculature is achieved primarily by thoracic rather than by abdominal pumping.)
13. The bees were heated in warm air from a hair dryer and then allowed to cool to near T_A while the body temperature (T_B) was observed every 30 seconds. The linear regression of the decrease in T_{Th} for ten animals is described by: $\log (T_{Th} - T_A) = 1.48 - 0.27$ minutes.
14. The rate of cooling of a 40-mg thorax, having a specific heat of about 0.8 cal per gram per degree Celsius, was about 17°C per minute when its temperature was 27°C above T_A. In order for T_{Th} to remain at 27°C above T_A, the necessary heat input was therefore equal to: 17°C per minute × 0.04 g × 0.8 cal per gram per degree Celsius = 0.54 cal per minute.
15. It is assumed (i) that the amount of heat produced in the abdomen of the 0.12-g animal is negligible in comparison with that produced in the thorax and (ii) that the utilization of 1 cm³ of O_2 yields 4.8 cal (0.54 cal per minute × 0.12 g × 60 minutes × 1 cm³ of O_2 per 4.8 cal = 56 cm³ of O_2 per gram per hour).
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22 March 1971; revised 29 July 1971

Initiation of Protein Synthesis at an Unusual Position in an Immunoglobulin Gene?

Abstract. *The amino acid sequence of urinary β_2-microglobulin has been partially determined and found to be related to the constant region of IgG immunoglobulin heavy chain. β_2-Microglobulin is present in normal individuals. Its gene may have evolved from an immunoglobulin gene by the use of an unusually located start signal for initiating synthesis of the polypeptide.*

Large deletions have been described in the genes controlling the synthesis of myeloma or myeloma-like immunoglobulins (*1*). The details of one of these deletions led Smithies *et al.* (*2*) to propose that all of the currently documented examples might be the consequence of DNA breakage and its nonhomologous repair. One of the possible outcomes of the breakage and repair events considered was that a start signal different from the normal one might be used to initiate protein synthesis in the neighborhood of a deletion. The data presented in this report suggest that this can occur.

In the course of a search for new examples of deletions in immunoglobulins and a general survey of urinary proteins of low molecular weight we determined the partial amino acid sequences of four proteins. Two were Bence Jones proteins with starch-gel electrophoretic mobilities indicating that they might be smaller than usual. The other two were not thought to be related to immunoglobulins although they had been isolated from a myeloma patient with severe renal tubular malfunction (*3*). One of these two was a retinol (vitamin A)-binding protein (*4*); the other was immunologically identical (*4*)

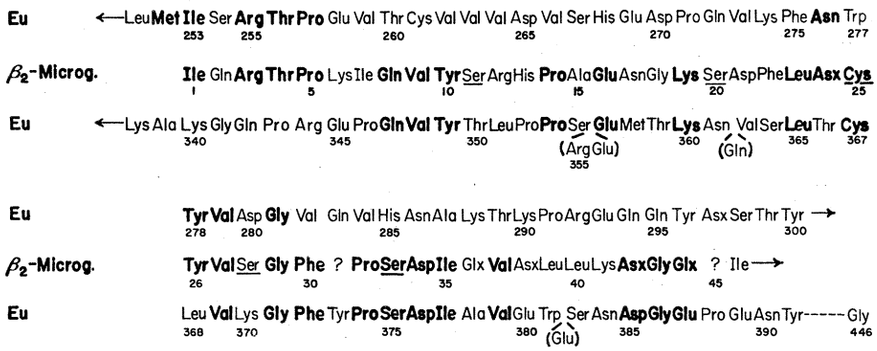

Fig. 1. A comparison in two different alignments of the partial sequence of β_2-microglobulin with parts of the constant region of the heavy polypeptide chain of the IgG_1 immunoglobulin Eu (*8*). Positions of identity are in boldface. The amino acids in parentheses are from the Eu sequence but are omitted from the comparisons because they appear to have no counterparts in β_2-microglobulin. The key to the three-letter code and the underlining is given in (*14*). The residues that are identical in Eu and β_2-microglobulin are also the same in the IgG_4 immunoglobulin Vin (*9*).

to the β_2-microglobulin of unknown function that was first characterized by Berggård and Bearn (*5*). To our surprise, the sequence of the first 12 residues of the β_2-microglobulin determined in a trial experiment suggested that the protein was related to two parts of the heavy chain of immunoglobulin G (IgG). Another sample of β_2-microglobulin was therefore prepared from the urine of a different patient who had recently received a kidney transplant (but did not have multiple myeloma). A sequence for 44 of the first 46 residues was determined for this protein in two independent experiments in which an Edman-Begg sequenator was used (*6*) and the procedures of Smithies *et al.* (*7*).

The partial amino acid sequence determined for the β_2-microglobulin is given in Fig. 1 where it is compared with sequences in the constant region of the heavy chain of the IgG_1 immunoglobulin Eu determined by Edelman *et al.* (*8*). Residues that are identical in Eu and β_2-microglobulin are shown in boldface type; the same residues also occur at all of these positions in the IgG_4 immunoglobulin Vin (*9*). Inspection of these sequences shows that four of the first five residues of the β_2-microglobulin are the same as those in Eu at positions 253 and 255–257. Position 252 in Eu is the relatively uncommon amino acid methionine, the codon for which can serve under suitable circumstances as the start signal for the initiation of protein synthesis (*10*). These findings are compatible with the start signal and the first five amino acids of β_2-microglobulin having evolved from the region 252 to 257 of an IgG-like gene. The residues in β_2-microglobulin at positions 8 to 10 are the same as the Eu residues at positions 347 to 349. This region is 87 residues farther along the sequence of Eu, suggesting that a large deletion in an IgG-like gene may have occurred during the evolution of the β_2-microglobulin gene (the reverse direction for this evolution involving an insertion cannot be excluded but seems much less likely).

Table 1. Comparison of Berggård and Bearn's observed (*5*) amino acid composition of β_2-microglobulin with that expected from the present sequence data extended according to the hypothesis proposed (*14*).

Amino acid	Residues (No.)	
	Observed	Expected
Asp	12	12
Thr	5	5
Ser	10	13
Glu	11	9
Pro	5	6
Gly	3	6
Ala	2	2
Cys	2	2
Val	7	7
Met	1	1
Ile	5	4
Leu	7	9
Tyr	6	5
Phe	5	5
His	4	4
Trp	2	1
Lys	8	7
NH_3	9	8–13
Arg	5	3
Unknown		2
Total (excluding NH_3)	100	103

Residues at 16 more of the 44 known β_2-microglobulin positions are found in Eu at positions compatible with the same large deletion together with three other small deletions (Fig. 1). Thus more than half of the known residues in β_2-microglobulin correspond to parts of the constant region of Eu and Vin (IgG_1 and IgG_4) heavy chains. Comparable data for IgG_2 and IgG_3 heavy chains are limited to 17 and 18 residues respectively (*11*), but at these positions all four IgG immunoglobulins are the same, having six residues identical with β_2-microglobulin. Of 18 residues known in these regions for immunoglobulin M (IgM) (*12*), only one is the same as β_2-microglobulin (compared to 10 of the comparable 18 IgG positions). No sequence data are yet available in these regions for other classes of immunoglobulins. Up to now provisional tests with antiserums have been relatively uninformative in the context of homologies since β_2-microglobulin does not react positively with antiserums specific for the heavy chains of IgG, IgM, IgA, IgD, or IgE or with antiserums against kappa or lambda light chains, or against the J chain, or the transport piece of secretory IgA (*4*).

W. M. Fitch compared our partial amino acid sequence of β_2-microglobulin with that of the heavy chain of Eu using computer programs he has already described (*13*). His tests, based on comparisons of sequences whose length is 20 residues, showed that, even without postulating any gaps, the probability is less than 1 in 10^3 that β_2-microglobulin would by chance be as similar to Eu as our data indicate it to be. We consequently conclude that the β_2-microglobulin gene is evolutionarily related to an immunoglobulin gene.

Several simple schemes for the evolution of the present-day β_2-microglobulin gene are readily imagined. The gene may have evolved from an ancestor of the present-day IgG genes primarily as a consequence of mutation or mutations leading to the formation of a valid initiator at a position equivalent to 339 in the Eu sequence; this scheme implies that the resemblance of the first few amino acids of β_2-microglobulin to the Eu region 253 to 257 is fortuitous. A second scheme is that the primary evolutionary event was a massive deletion of 87 amino acids in an Eu-like ancestral gene from positions 260 through 346, with the deletion enabling a methionine codon already at position 252 to

serve as a valid initiator. We prefer this second evolutionary scheme but cannot prove that it provides a better explanation of the data than the other. Clearly we cannot exclude the possibility that future information will show the β_2-microglobulin gene to be more closely related to the gene for the constant region of one of the immunoglobulin classes not yet sequenced than it is to the IgG genes.

The two evolutionary schemes considered have in common the hypothesis that the β_2-microglobulin gene was derived from an IgG-like gene. The molecular size and complete amino acid composition of β_2-microglobulin can accordingly be predicted approximately by adding to the presently determined partial sequence of 46 amino acids the remainder of the constant region of IgG_1 from position 390 (where our sequence data end) to the carboxy terminus of IgG_1 at position 446. The predicted values can then be compared with the data of Berggård and Bearn (*5*) observed experimentally. Table 1 shows the comparison. The agreement is satisfactory when allowance is made for the expectation that mutations will have occurred in both descendants subsequent to the first formation of the β_2-microglobulin gene from its presumed IgG-like ancestor. The hypothesis is consequently supported by this independent test. A determination of the complete sequence of β_2-microglobulin should provide a more stringent test.

The data of Berggård and Bearn (*5*) show that the β_2-microglobulin gene is present and translated in normal individuals, since the protein was found in urine and plasma from ten healthy individuals and in five presumed normal samples of cerebrospinal fluid. The questions therefore arise as to whether the DNA corresponding to the beginning part of the immunoglobulin heavy chain gene which we postulate to be the progenitor of the β_2-microglobulin gene is still in the genome of present-day individuals, and if so whether it is transcribed into RNA and translated into protein with or without a variable region attached to it. What function, if any, β_2-microglobulin has in the immune system is likely to depend on the answers to these questions. In any case, a careful search for the function and species distribution of this interesting protein appears to be warranted.

In conclusion, the partial amino acid sequence of a normal human protein, β_2-microglobulin, is compatible with the evolution of its gene from the carboxy terminal portion of an immunoglobulin constant region gene as the result of the use of a new start signal for initiating synthesis of the polypeptide.

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15. We thank Dr. Walter M. Fitch, University of Wisconsin, for help and advice in making the amino acid sequence comparisions. Paper 1507 from the Laboratory of Genetics, University of Wisconsin. Supported in part by NSF GB-4362, NIH GM-1522 and NIH HE-07495.

27 September 1971; revised 18 November 1971 ■

Cellular Site of Glucocorticoid-Receptor Complex Formation

Abstract. *The cellular site of binding of dexamethasone by specific glucocorticoid receptors in cultured hepatoma cells was investigated with the use of certain mercurials.* p-*Chloromercuribenzene sulfonate and* p-*chloromercuribenzoate inhibit the binding of steroid by receptors in cell-free extracts, but they allow the steroid-receptor complex to form in whole cells. In contrast, $HgCl_2$ inhibits binding both in extracts and cells. Since both organic mercury compounds, unlike $HgCl_2$, do not readily enter intact cells, it appears that the specific steroid binding occurs inside the cell rather than at the cell membrane.*

The first step in the cellular action of steroid hormones in many tissues appears to be intracellular binding of the hormone by cytoplasmic receptors (*1*). The latter, upon complexing with the steroid, are thought to migrate to the nucleus to initiate the characteristic biologic response (*2*). However, many hormones act on the cell surface (*3*). Furthermore, considerable evidence suggests that steroids do interact with and directly modify membranes (*4*) and that, in amphibian oocytes, progesterone can activate protein synthesis by acting on the cell surface (*5*). We therefore thought it important to investigate further, using a different approach, whether the steroid receptors are truly cytoplasmic or merely appear so because they, like many surface proteins (*6*), are released from membranes during cell fractionation.

We have been studying the glucocorticoid-specific receptor interactions in cultured rat hepatoma (HTC) cells, and report here evidence that dexamethasone binding to these receptors takes place inside the cell. For these experiments, we have taken advantage of the findings that mercurials inhibit the specific dexamethasone binding by cytoplasmic extracts of HTC cells (*7*), and that under certain conditions some of these inhibitors do not readily enter