

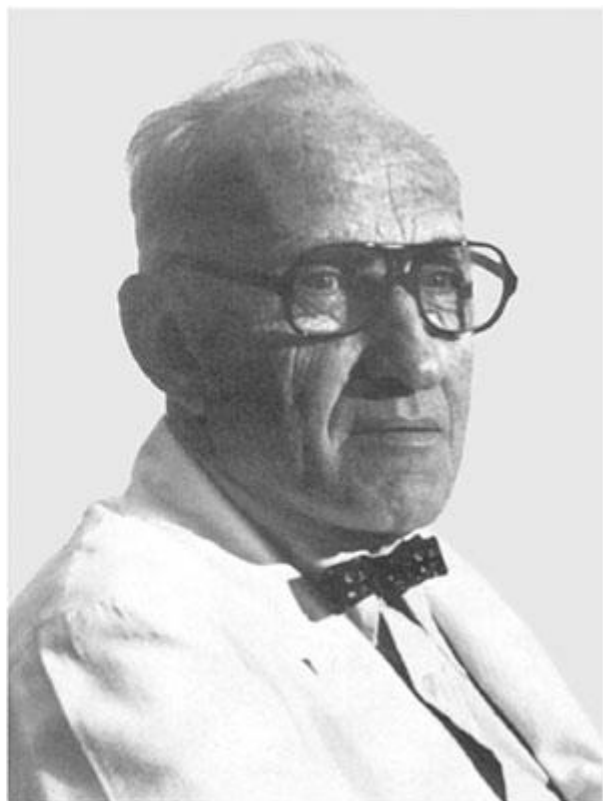
## History of Immunoglobulin molecules

### Snapshots in the history of Immunoglobulin molecules



1939

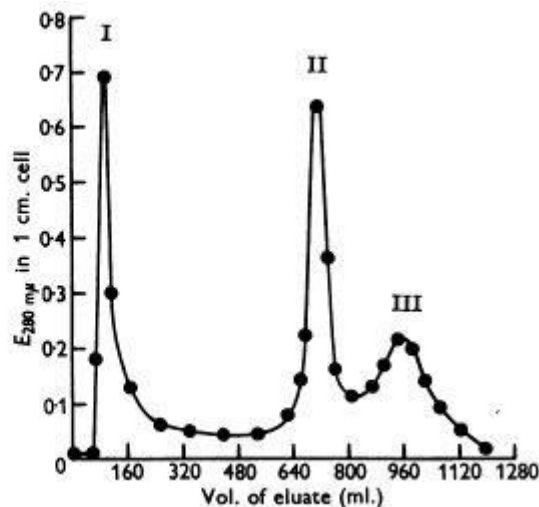
#### **gamma-Globulin**



Tiselius and Kabat in 1939 showed that antibodies belong to the  $\gamma$ -globulin fraction of serum proteins

1959

#### **Three Fractions**



Porter digested  $\gamma$ -globulins with papain, a proteolytic enzyme, and recovered 3 fractions: Fractions I and II of molecular weights between 50 and 55KDa retained the antigen binding capacity, whereas fraction III, of 80 KDa was crystallizable, and had a higher carbohydrate content ([Porter RR, Biochem J. 73:119-127, 1959](#)).

1961

## Heavy and Light chains

HYPOTHETICAL RELATIONS BETWEEN TYPES OF POLYPEPTIDE CHAINS AND PROPERTIES OF  $\gamma$ -GLOBULINS

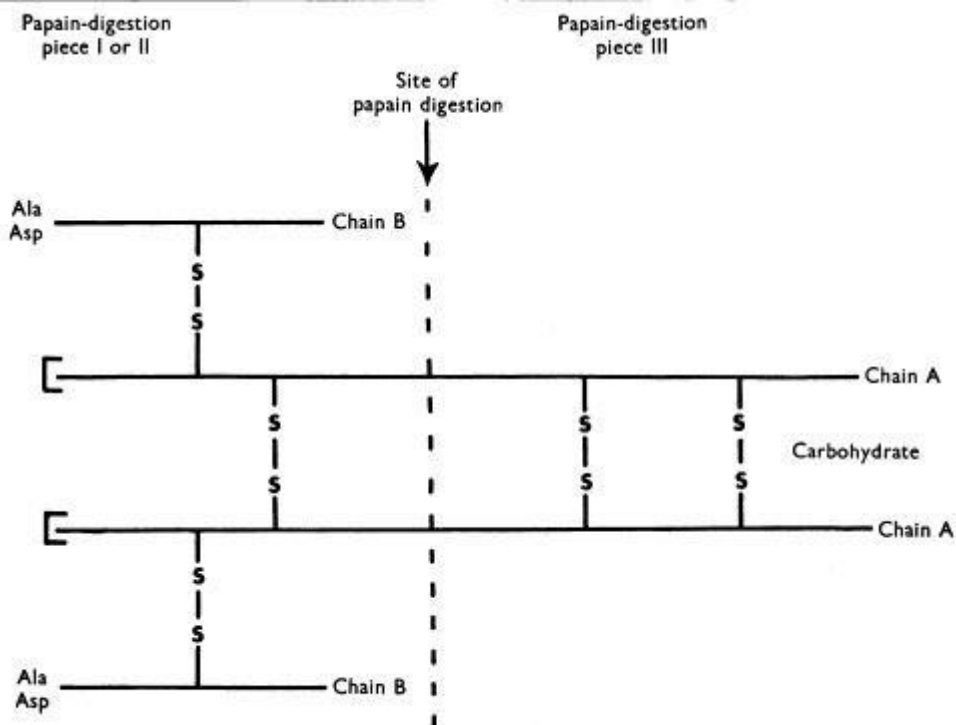
$\gamma$ -Globulin class		Type and number of chains	Properties assigned to H chains	Properties assigned to L chains
Ultra-centrifugal	Immuno-electrophoretic			
7S	$\gamma_2$ $\gamma_{1A}$	Small number of L and H* chains	Complement fixation, Skin fixation Placental passage (? Immunologic specificity)	Antibody specificity. Heterogeneity. Antigenic cross-reactivity with other $\gamma$ -globulins.
19S	$\gamma_{1M}$	Large number of L and H* chains	Complement fixation (? Immunologic specificity)	Antibody specificity. Heterogeneity. Antigenic cross-reactivity with other $\gamma$ -globulins.
3.4S	Bence-Jones	L chains†	...	Antigenic cross-reactivity with other $\gamma$ -globulins. Reversible temperature dependent solubility properties.

\*  $\gamma_2$ -globulins,  $\gamma_{1A}$ -globulins, and  $\gamma_{1M}$ -globulins appear to possess different kinds of H chains (see text).  
† Most Bence-Jones proteins have molecular weights consistent with the presence of two L chains.

Edelman and Poulik reported that rabbit 7S  $\gamma$ -globulins and human myeloma proteins reduced in strong urea solutions and alkylated, separated into heavy (H) and light (L) chains bound by disulfide bonds ([Edelman GM and Poulik MD, J Exp Med. 113:861-884, 1961](#))

1963

## Y Structure



Scheme 1. Diagrammatic structure of rabbit  $\gamma$ -globulin (Porter, 1962).

Porter and colleagues proposed the basic Y structure of four polypeptide chains and 5 interchain disulfide bonds ([Fleischman JB et al., Biochem J. 88:220-228, 1963](#))

1965

## V and C Regions

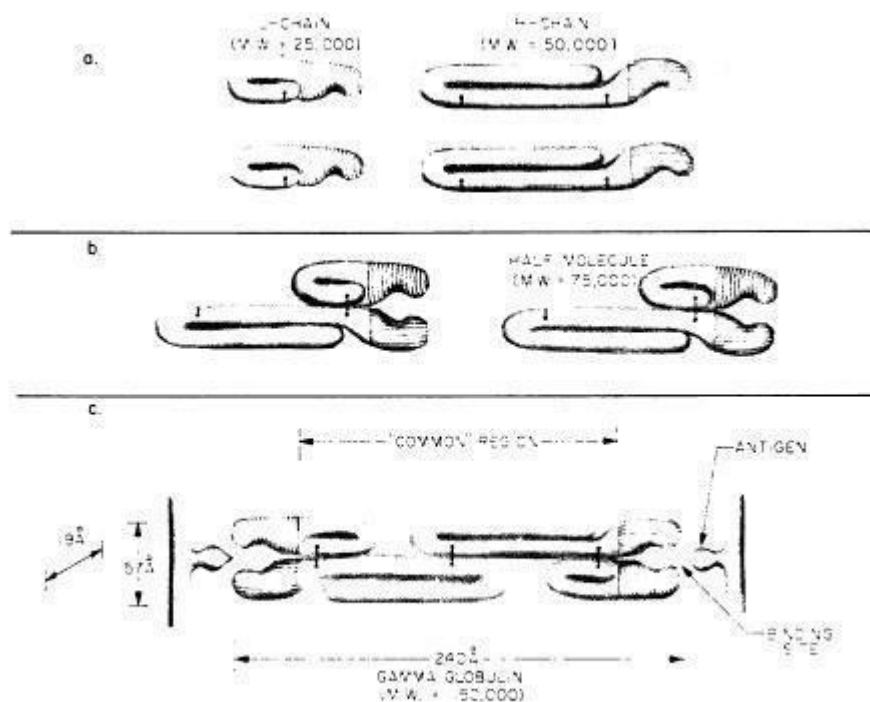


FIG. 1.—Diagrammatic representation of the multiple chain structure of rabbit gamma globulin (see text). Covalent, interchain disulfide linkages (●—●) serve to stabilize the complex structure after formation.

Dreyer and Bennett proposed that the V and C regions must be the products of different genes ([Proc Nat Acad Sci USA 54: 864-869, 1965](#))

## IgA

TABLE II  
Effect on Anti-B Agglutinins after Absorption with Specific Antisera

Sample	Saline control	Prior absorption with		
		Anti- $\gamma_1$ A	Anti- $\gamma_2$ S	Anti- $\gamma_1$ M
L. T. saliva.....	3+	0	2+	3+
J. C. saliva.....	3	Tr.	3	3
D. D. saliva.....	4	0	1+	4
L. C. saliva.....	4	0	1+	4
S. Z. colostrum.....	3+	0	3+	3+
L. D. colostrum.....	4	0	3	4
L. H. serum*.....	3	2	2+	0

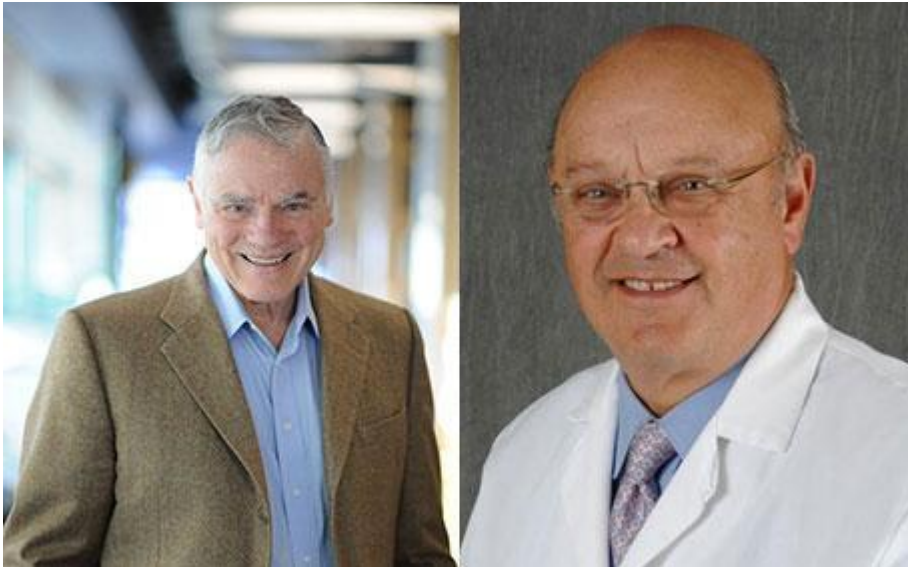
\* Serum completely lacked  $\gamma_1$ A; agglutinins found only in 19S region on density gradient ultracentrifugation.

Tomasi and coworkers demonstrated that IgA present in saliva and colostrum is produced locally and secreted as a dimer or trimer by ([Tomasi TB et al., J Exp Med 121:101-124, 1965](#)) and Newcomb and coworkers demonstrated the existence of the secretory piece ([Newcomb RW et al., J Immunol](#)

[101:905-913, 1968](#)).

1968

## **Lambda chain**



Hood and Ein confirmed that the Lambda chain is encoded by two separate genes that are expressed as a single polypeptide chain ([Nature 220:764-767, 1968](#))

1969

## **Variable and Constant Regions**



EU C <sub>L</sub> (RESIDUES 109-214)										110										120									
EU C <sub>H1</sub> (RESIDUES 119-220)										THR	VAL	ALA	ALA	PRO	SER	VAL	PHE	ILE	PHE	PRO	PRO	SER							
EU C <sub>H2</sub> (RESIDUES 234-341)										SER	THR	LYS	GLY	PRO	SER	VAL	PHE	PRO	LEU	ALA	PRO	SER							
EU C <sub>H3</sub> (RESIDUES 342-446)										LEU	LEU	GLY	GLY	PRO	SER	VAL	PHE	LEU	PHE	PRO	PRO	LYS							
										GLN	PRO	ARG	GLU	PRO	GLN	VAL	TYR	THR	LEU	PRO	PRO	SER							
										130																			
ASP	GLU	GLN	-	-	LEU	LYS	SER	GLY	THR	ALA	SER	VAL	VAL	CYS	LEU	LEU	ASN	ASN	PHE										
SER	LYS	SER	-	-	THR	SER	GLY	GLY	THR	ALA	ALA	LEU	GLY	CYS	LEU	VAL	LYS	ASP	TYR										
PRO	LYS	ASP	THR	LEU	MET	ILE	SER	ARG	THR	PRO	GLU	VAL	THR	CYS	VAL	VAL	VAL	ASP	VAL										
ARG	GLU	GLU	-	-	MET	THR	LYS	ASN	GLN	VAL	SER	LEU	THR	CYS	LEU	VAL	LYS	GLY	PHE										
140										150																			
TYR	PRO	ARG	GLU	ALA	LYS	VAL	-	-	GLN	TRP	LYS	VAL	ASP	ASN	ALA	LEU	GLN	SER	GLY										
PHE	PRO	GLU	PRO	VAL	THR	VAL	-	-	SER	TRP	ASN	SER	-	GLY	ALA	LEU	THR	SER	GLY										
SER	HIS	GLU	ASP	PRO	GLN	VAL	LYS	PHE	ASN	TRP	TYR	VAL	ASP	GLY	-	VAL	GLN	VAL	HIS										
TYR	PRO	SER	ASP	ILE	ALA	VAL	-	-	GLU	TRP	GLU	SER	ASN	ASP	-	GLY	GLU	PRO	GLU										
										160										170									
ASN	SER	GLN	GLU	SER	VAL	THR	GLU	GLN	ASP	SER	LYS	ASP	SER	THR	TYR	SER	LEU	SER	SER										
-	VAL	HIS	THR	PHE	PRO	ALA	VAL	LEU	GLN	SER	-	SER	GLY	LEU	TYR	SER	LEU	SER	SER										
ASN	ALA	LYS	THR	LYS	PRO	ARG	GLU	GLN	GLN	TYR	-	ASP	SER	THR	TYR	ARG	VAL	VAL	SER										
ASN	TYR	LYS	THR	THR	PRO	PRO	VAL	LEU	ASP	SER	-	ASP	GLY	SER	PHE	PHE	LEU	TYR	SER										
										180										190									
THR	LEU	THR	LEU	SER	LYS	ALA	ASP	TYR	GLU	LYS	HIS	LYS	VAL	TYR	ALA	CYS	GLU	VAL	THR										
VAL	VAL	THR	VAL	PRO	SER	SER	SER	LEU	GLY	THR	GLN	-	THR	TYR	ILE	CYS	ASN	VAL	ASN										
VAL	LEU	THR	VAL	LEU	HIS	GLN	ASN	TRP	LEU	ASP	GLY	LYS	GLU	TYR	LYS	CYS	LYS	VAL	SER										
LYS	LEU	THR	VAL	ASP	LYS	SER	ARG	TRP	GLN	GLU	GLY	ASN	VAL	PHE	SER	CYS	SER	VAL	MET										
										200										210									
HIS	GLN	GLY	LEU	SER	SER	PRO	VAL	THR	-	LYS	SER	PHE	-	-	ASN	ARG	GLY	GLU	CYS										
HIS	LYS	PRO	SER	ASN	THR	LYS	VAL	-	ASP	LYS	ARG	VAL	-	-	GLU	PRO	LYS	SER	CYS										
ASN	LYS	ALA	LEU	PRO	ALA	PRO	ILE	-	GLU	LYS	THR	ILE	SER	LYS	ALA	LYS	GLY												
HIS	GLU	ALA	LEU	HIS	ASN	HIS	TYR	THR	GLN	LYS	SER	LEU	SER	LEU	SER	PRO	GLY												

Edelman and coworkers reported the first complete sequence of a  $\gamma$ G immunoglobulin molecule and demonstrated the existence of variable (V) and constant (C) regions in the H and L chains ([Edelman GM et al., Proc Nat Acad Sci USA 63:78-85, 1969](#))

1972

## Nobel Prize - 1972



Edelman and Porter shared the Nobel Prize in Medicine in 1972 “for their discoveries concerning the chemical structure of antibodies”

[Gerald M. Edelman – Facts](#). *Nobelprize.org*. Nobel Media AB 2014.

[Rodney R. Porter – Facts](#). *Nobelprize.org*. Nobel Media AB 2014.

1974

## **Monomers**

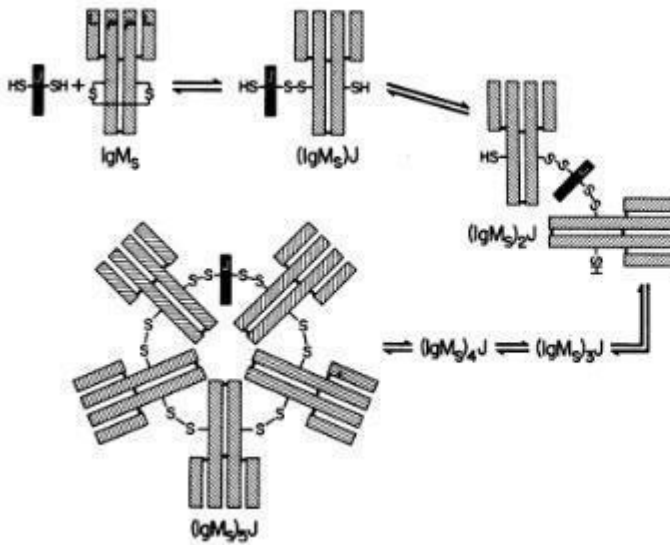
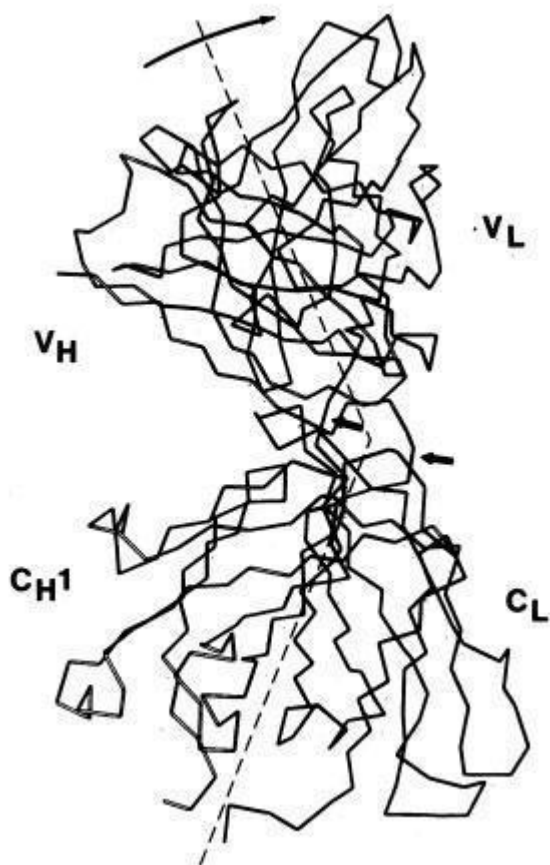


FIG. 5. The clasp model of J linkage in pentamer IgM and the postulated disulfide exchanges leading to its formation. IgM<sub>s</sub> = monomer of pentameric IgM.

Koshland and coworkers demonstrated that the monomers of the polymeric IgM and IgA are linked by the J chain in a clasp way ([Halpern MS and Koshland ME. Nature 228:1276-1278, 1970](#); [Chapuis RM, Koshland ME, Proc Nat Acad Sci 71:657-661, 1974](#))

### 3D Structure

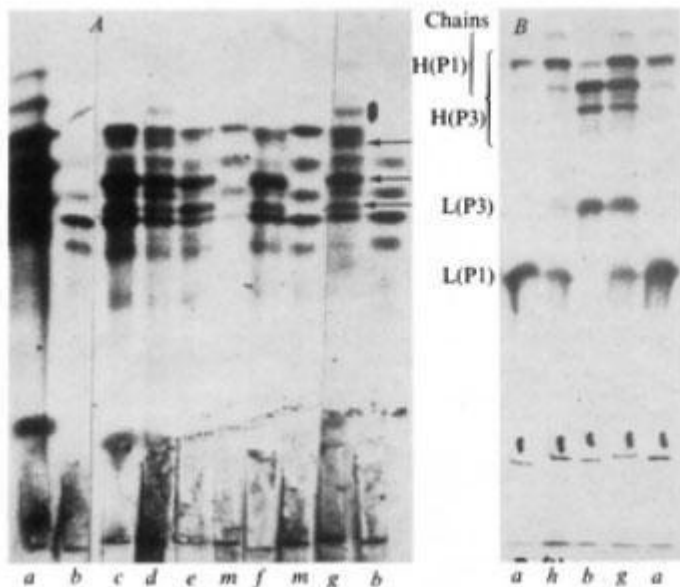




Poljak and colleagues described the three-dimensional structure of IgG(I) myeloma protein ([Poljak et al., Proc Nat Acad Sci 71. 3440-3444, 1974](#)).

1975

## Monoclonal antibodies

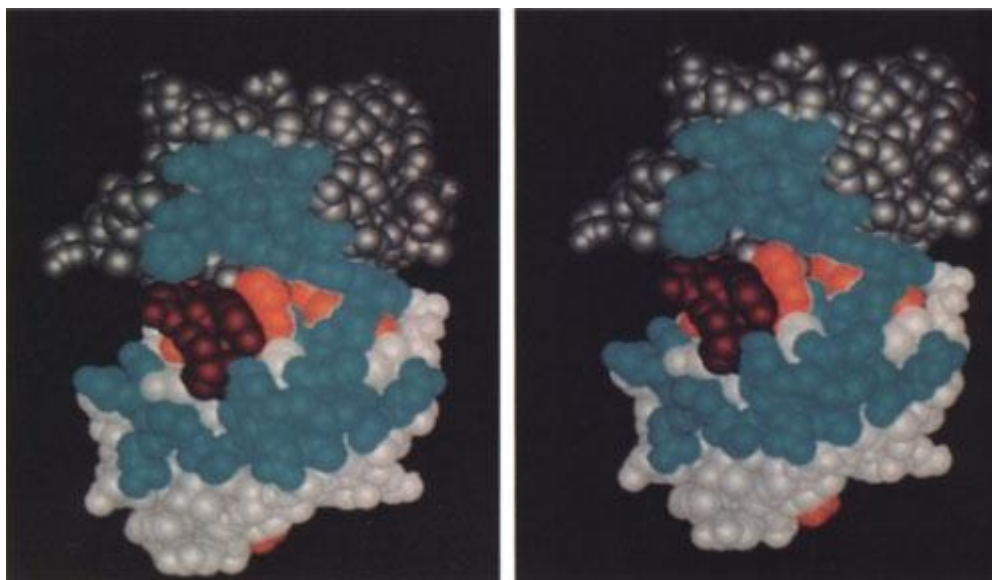


**Fig. 1** Autoradiograph of labelled components secreted by the parental and hybrid cell lines analysed by IEF before (A) and after reduction (B). Cells were incubated in the presence of  $^{14}\text{C}$ -lysine<sup>14</sup> and the supernatant applied on polyacrylamide slabs. A, pH range 6.0 (bottom) to 8.0 (top) in 4 M urea. B, pH range 5.0 (bottom) to 9.0 (top) in 6 M urea; the supernatant was incubated for 20 min at 37 °C in the presence of 8 M urea, 1.5 M mercaptoethanol and 0.1 M potassium phosphate pH 8.0 before being applied to the right slab. Supernatants from parental cell lines in: a, P1Bul; b, P3-X67Ag8; and m, mixture of equal number of P1Bul and P3-X67Ag8 cells. Supernatants from two independently derived hybrid lines are shown: e-f, four subclones from Hy-3; g and h, two subclones from Hy-B. Fusion was carried out<sup>14</sup> using  $10^6$  cells of each parental line and 4,000 haemagglutination units inactivated Sendai virus (Searle). Cells were divided into ten equal samples and grown separately in selective medium (HAT medium, ref. 6). Medium was changed every 3 d. Successful hybrid lines were obtained in four of the cultures, and all gave similar IEF patterns. Hy-B and Hy-3 were further cloned in soft agar<sup>15</sup>. L, Light; H, heavy.

Kohler and Milstein ([Nature 256: 495-497, 1975](#)) reported that the fusion of a myeloma cell with a spleen specific antibody-producing cell results in a hybridoma that produces monoclonal antibodies against the specific antigen. Continuous culture of cloned hybrid cells allows the production of large amounts of monoclonal antibodies against the desired antigen.

1979

## Somatic Rearrangements

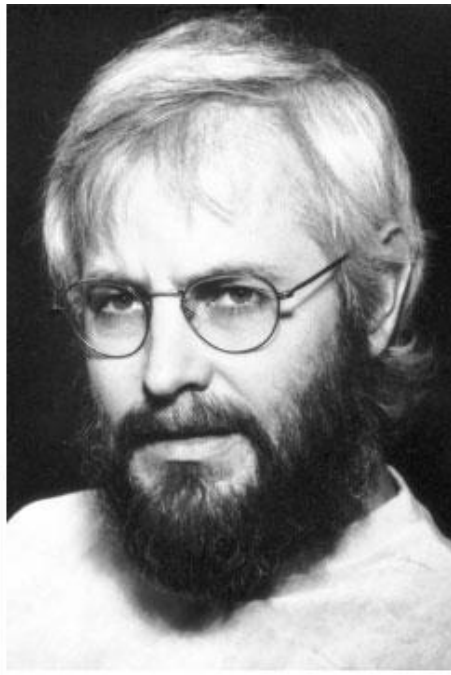


In the late 1970s, Tonegawa and colleagues in a series of elegant experiments demonstrated that

immunoglobulin V and C genes undergo somatic rearrangements to form the complete immunoglobulin gene ([Hozumi N, Tonegawa S, Proc Nat Acad Sci 73: 3628- 3632, 1976](#); [Brack C et al., Cell 15:1-14, 1978](#); [Sakano et al., Nature 277:627-633, 1979](#); [Sakano et al., Nature 280: 288-294, 1979](#); [Tonegawa S. Nature 302:575, 1983](#))

1984

## Nobel Prize - 1984



In 1984, Niels Jerne, Georges Kohler and Cesar Milstein were awarded with the Nobel Prize for their discovery of the hybridomas technology for the production of large amounts of monoclonal antibodies for experimental, analytical, diagnostic and therapeutic purposes.

[Niels K. Jerne – Facts](#). *Nobelprize.org*. Nobel Media AB 2014.

[Georges J.F. Köhler – Facts](#). *Nobelprize.org*. Nobel Media AB 2014.

[César Milstein – Facts](#). *Nobelprize.org*. Nobel Media AB 2014.

1987

## Nobel Prize - 1987



In 1987, Susumo Tonegawa was awarded with the Nobel Prize for his discoveries on the mechanisms of somatic rearrangement of the immunoglobulin genes.

[Susumo Tonegawa – Facts](#). *Nobelprize.org*. Nobel Media AB 2014.

### **Acknowledgement**

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