Infant B Cell Response Against Rotavirus and RSV: Analysis at the Single Cell Level

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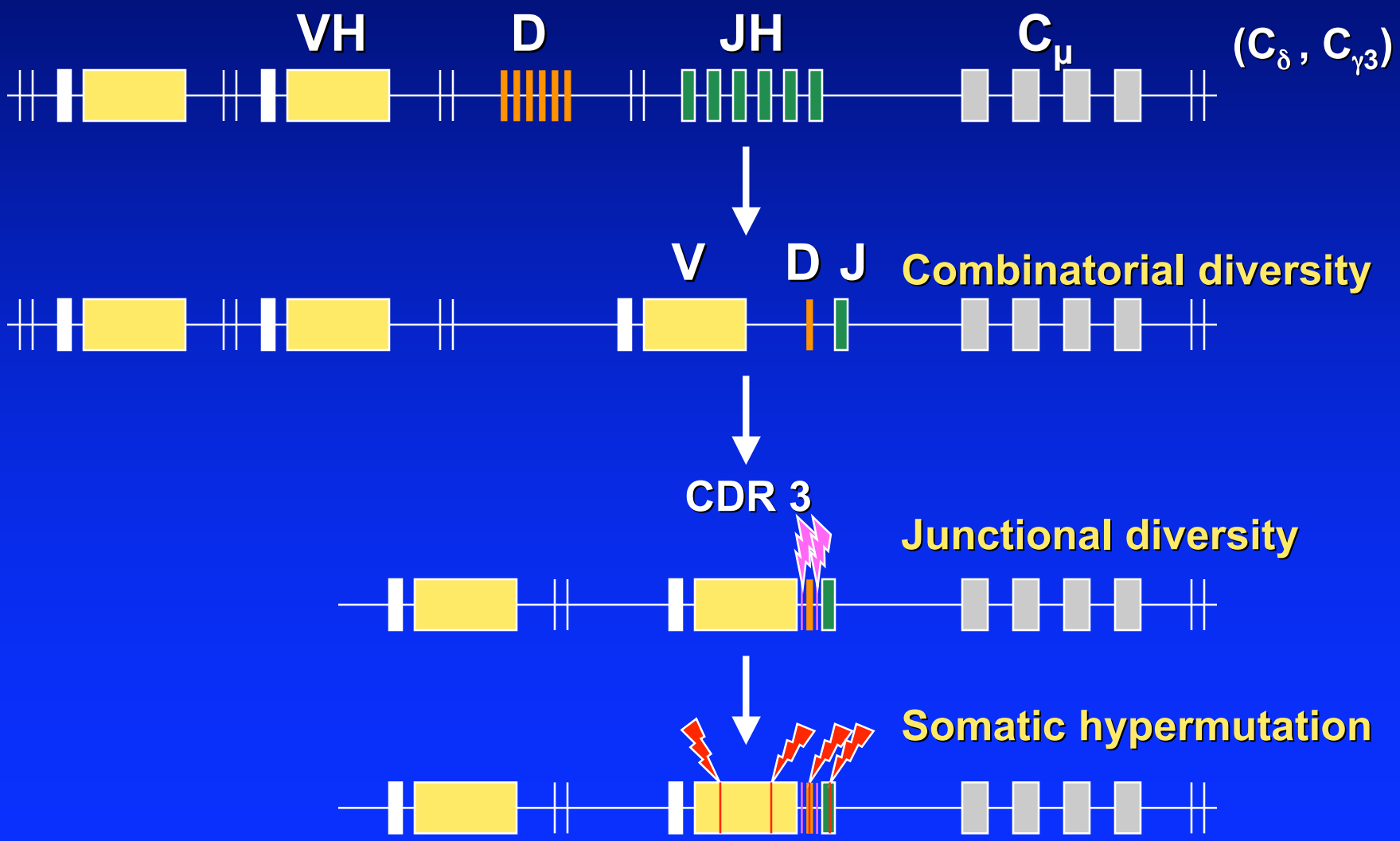
Summary of serum antibody responses RSV *cpts*-248/404 vaccine

Age (months)		Dosage	# Studied	# With antibody responses		
				Neut	F protein	G protein
15-59	sero+	10^5	11	0	0	0
6-24	sero-	10^4- 10^5	30	24 (80%)	25 (85%)	26 (87%)
3-5	naive	10^5	10	4 (44%)	7 (78%)	5 (56%)
1-2	naive (dose 1)	10^4- 10^5	24	0	0	1 (4%)
	(dose 2)	10^4- 10^5	22	5 (23%)	4 (18%)	7 (32%)

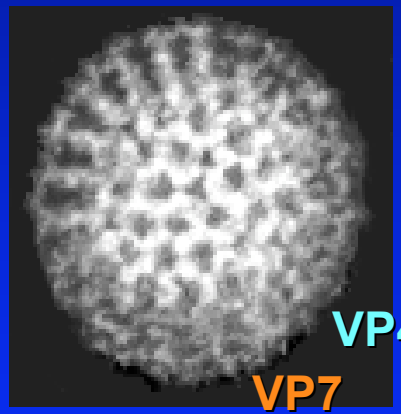
What is the molecular basis for the observed immaturity in the B cell response of human infants to viruses or virus vaccines?

Mechanisms of Antibody Diversity



Rotavirus reagents

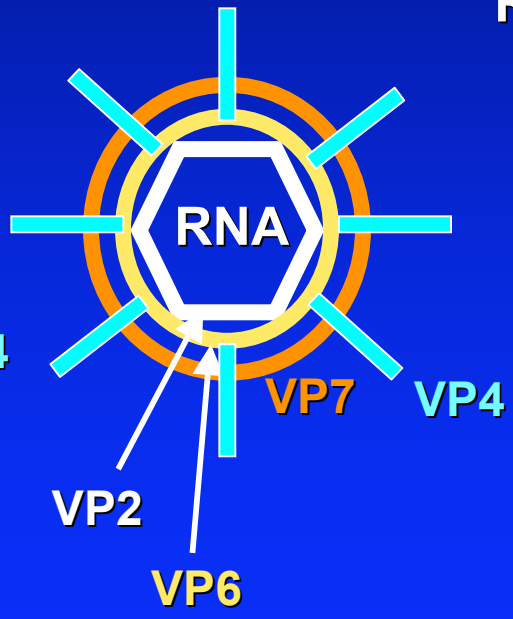
RV particle as seen by EM



75 nm

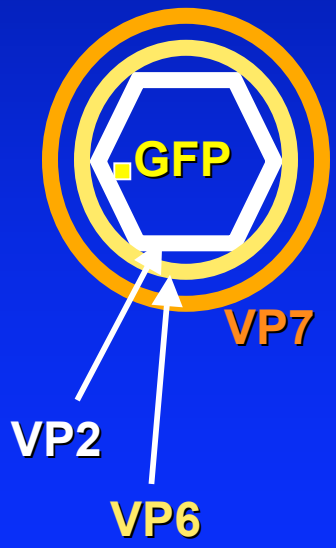
Linda Stannard

RV - Structure



Virus-like particles

RV 2/6/7 (VP7)



RV 2/6 (VP6)

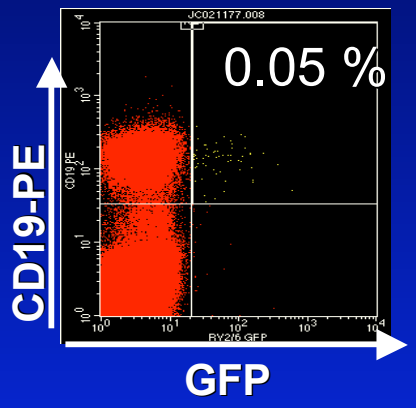
B Cell Methods

Anti-CD19-PE

B-cell

VLP-GFP

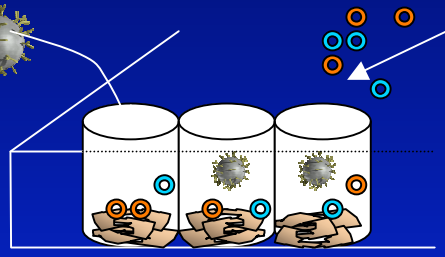
FACS



Double positive

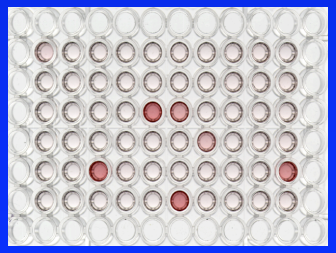
Expansion

rIL-2
rIL-4

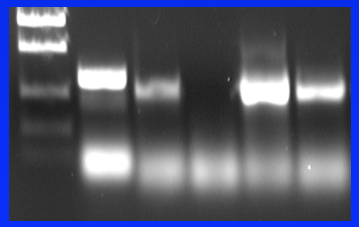


CD40L-expressing feeder cells

ELISA



RT-PCR

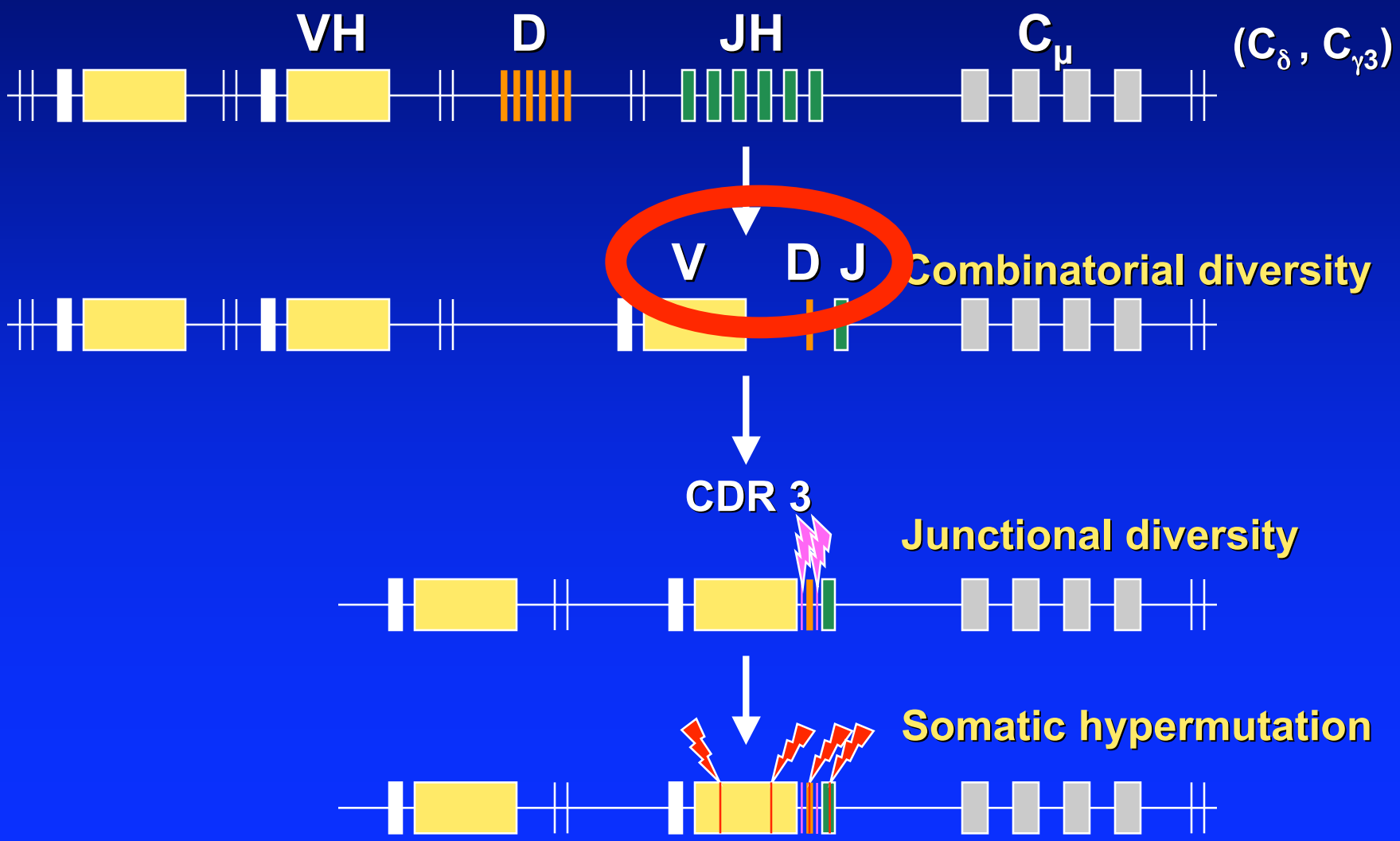


Sequencing

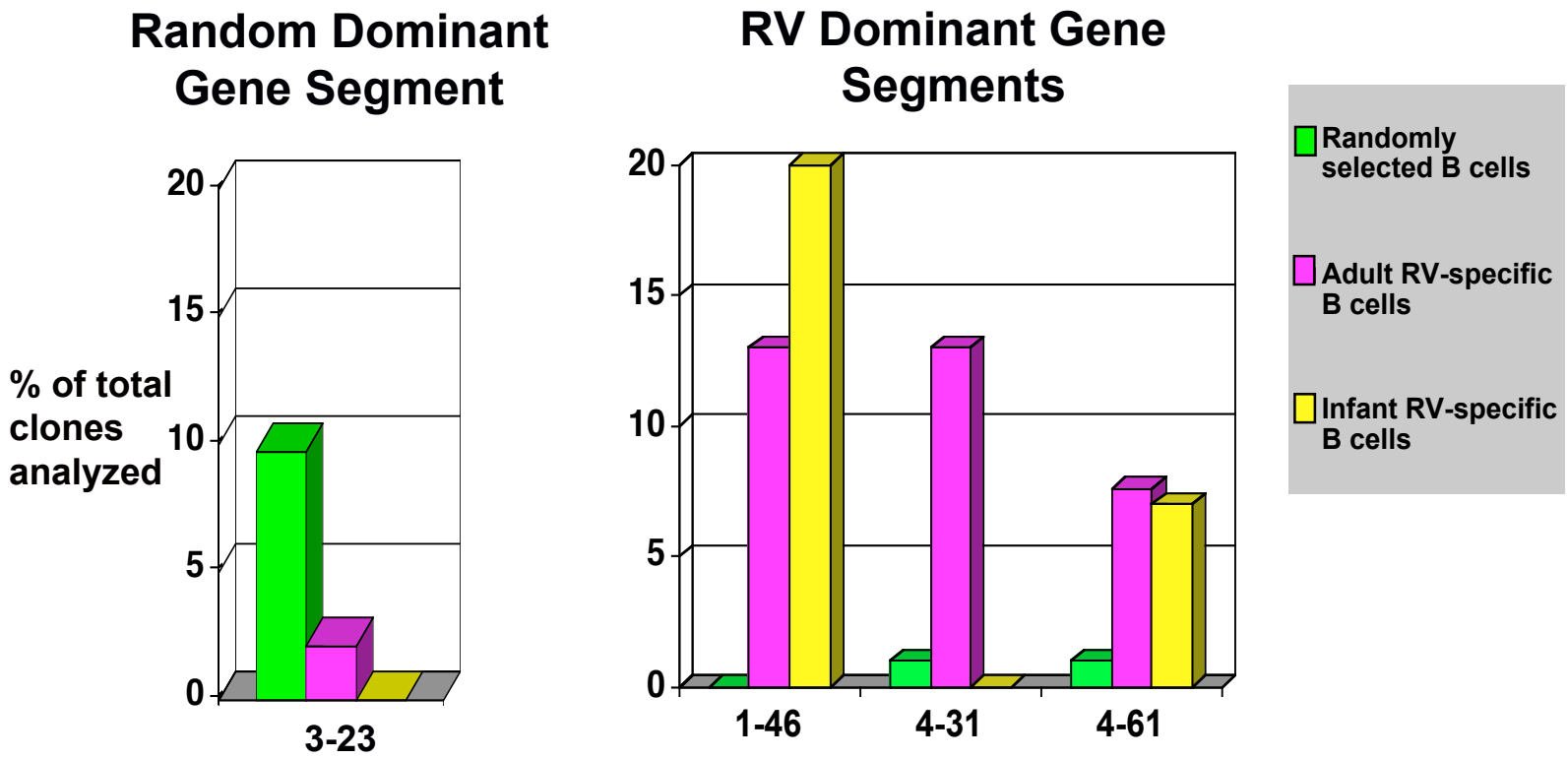
…CARLAARPLDYW…

Weitkamp et al. J Immunol Methods. 2003;275:223-37

Mechanisms of Diversity: I

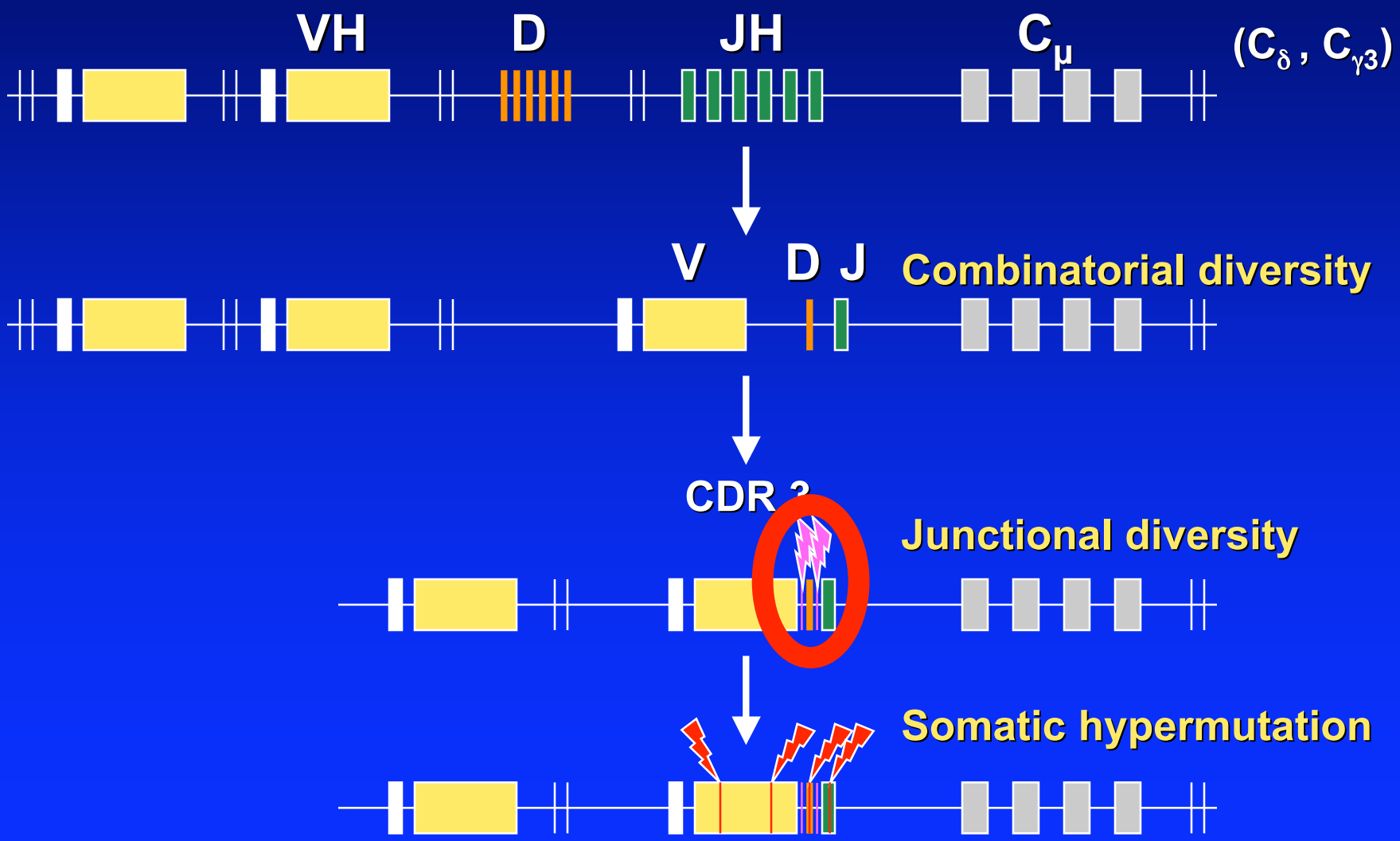


Three VH gene segments dominate the RV-specific repertoire



Weikamp et al, Journal of Immunology 2003

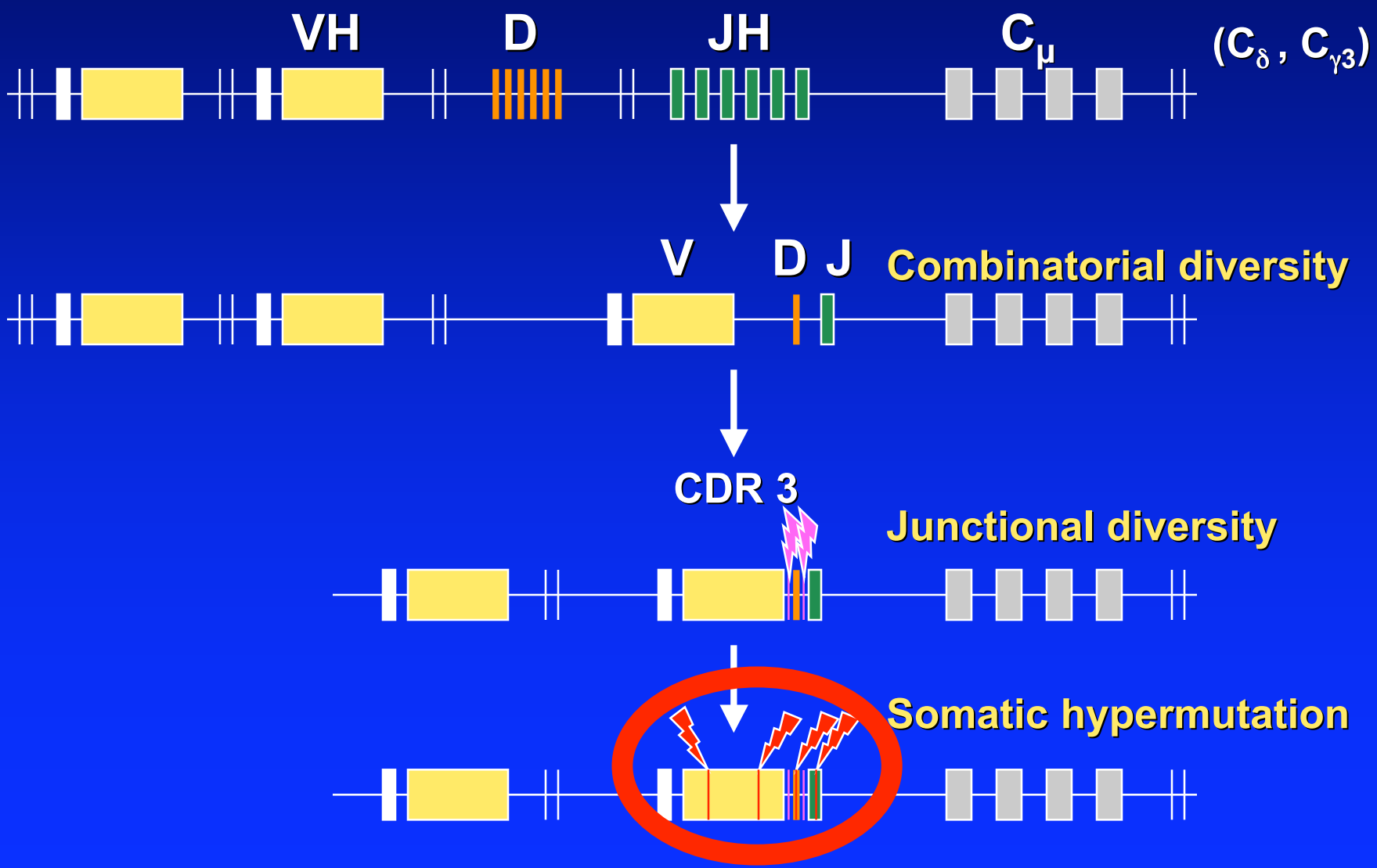
Mechanisms of Diversity: II



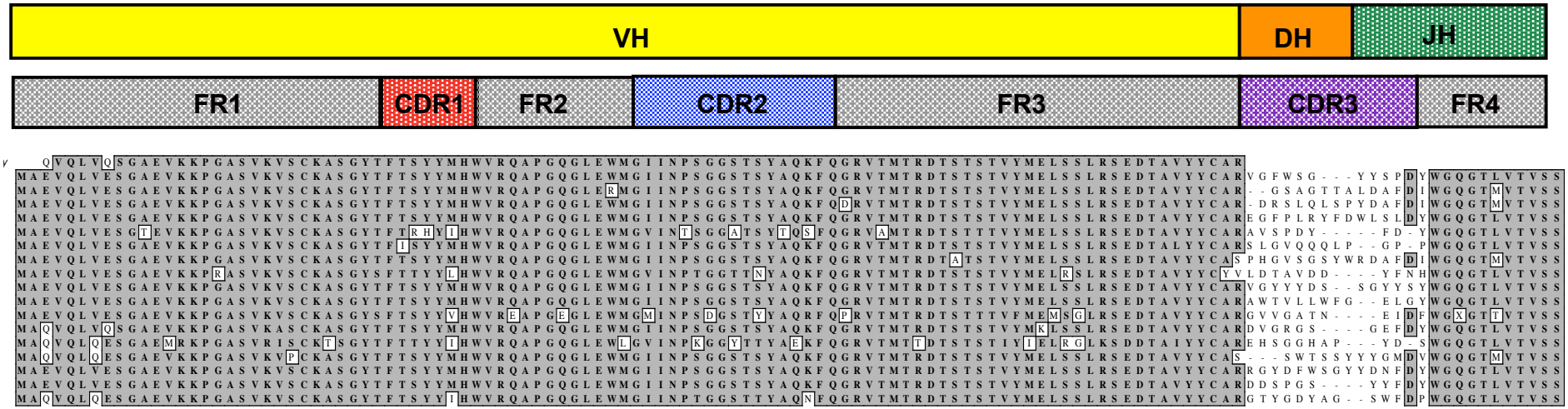
Junctional diversity, D segment length and HCDR3 lengths were similar in adults and infants

Type of donor	Mean number of amino acids altered on the N- or C-terminus of D	Mean amino acid length of D segment	Mean amino acid length of CDR3
Infant/RV+	5.0	5.3	14.4
Adult/RV+	5.5	4.4	13.7
Adult memory	7.2	5.8	14.1
Random B cells	5.5	5.4	13.9

Mechanisms of Diversity: III



Alignment of representative VH 1-46 rotavirus-specific antibody sequences



Infants demonstrated a significant lack in mutations within the HCDR3

Type of donor	Percent of D segments assignable by database alignment		Percent of JH segments lacking mutations	
Infant/RV+	93	p=0.022	100	p=0.024
Adult/RV+	56		80	
Adult memory	33		67	
Random B cells	64		88	

Infant RV-specific antibodies also exhibit a paucity of somatic mutations in VH sequences

Type of donor	HCDRs 1 and 2 Mutations		Mean % nucleotide change from germline
	Replacement	Silent	
Infant/RV+	0.2	0	0.3
Adult/RV+	2.1	0.5	2.7
	p=0.018		p=0.013
Adult memory	3.7	1.9	8.2
Random B cells	0.8	0.2	1.2

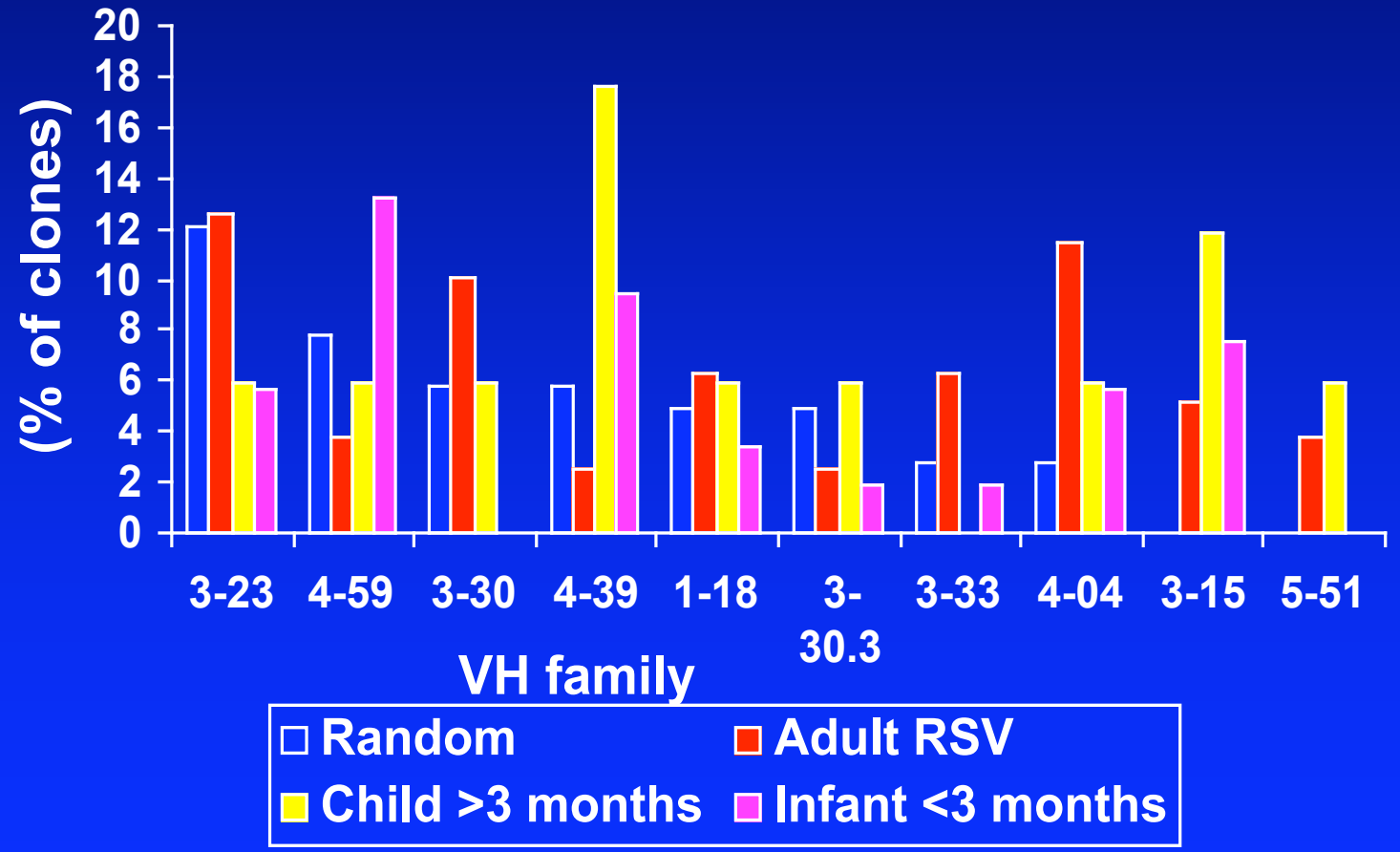
Summary I

RV-specific B cells exhibit a distinct bias in use of VH1/VH4 gene segments that differed markedly from randomly selected adult B cells.

The RV-specific B cell repertoires of infants and adults were highly related

The major difference between infant and adult B cell sequences is the lack of somatic mutations in infant sequences.

RSV-specific B cells use different dominant segments than random B cells



Young infants exhibit a striking paucity of somatic mutations in VH genes

Mean number of mutations or nucleotides in each VH segment stratified by age

	FR	CDR	N	P	D	J	CDR3 (aa)
Adult (n = 78)	3.1	1.7	10.1	0.6	1.9	0.7	15.9
≥ 3 mos (n = 17)	4.8*	2.7†	13.3	0.5	1.8	0.9	17.1
< 3 mos (n = 51)	0.9*	0.4†	9.1	0.6	1.2 §	0.5	16.9

** p = 0.002 vs. adults, < 0.05 vs. ≥ 3 months*

† p < 0.002 vs. adults, < 0.05 vs. ≥ 3 months

§ p < 0.05 vs. adults

Student's t-test, 2-tailed, unequal variance.

Lack of SHM in RSV-specific B cells following second infection in an infant

Nine clones were obtained from an 11 week old, former 29-week gestation infant with previous RSV infection at 3 weeks of age

Only 1 nucleotide was mutated in 3 clones.

Summary II

Infant antibody sequences specific for the two most common acute viral infections of infancy, RSV and rotavirus, share immunodominant gene segment usage with adults, but infant sequences lack somatic mutations.

Next questions:

1. Are infant B cells incapable of introducing mutations, or are infants simply lacking in prior exposure to antigen?

2. Does the lack of mutations have functional consequences for the antibodies of infants?

Ongoing studies

Transcriptional upregulation of enzymes involved in somatic mutation (AID, DNA polymerases)

Structure/function studies of antibodies

Mucosal versus regional B cells

Antigen specific repertoire of B cell subsets
naïve, IgD+ CD27+, memory

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