Yuen (*12*). Recent studies by Leitch *et al.* (*21*) and Yuen *et al.* (*22*) have shown that the decrease of α with depth plays a significant role in deep mantle convection and results in steady hot plumes and in convection cells with large aspect ratios. For multiple phase changes, depth-dependent α may allow deep mantle plumes to break through the closely clustered phase transitions. Otherwise, deep mantle plumes might be confined to the lower mantle and layered convection would prevail (*9, 13*).

Internal heating from radioactive decay is another important factor that helps to produce mantle diapirs. All of the results we have shown in this report are calculated with $R = 10$, nearly the chondritic value. In other numerical experiments without internal heating ($R = 0$), we find that pure basal heating would produce very steady plumes. These plumes cannot as easily be torn up into mantle diapirs as plumes in internally heated systems. Temperature-dependent viscosity causes thinner and faster plumes, which are dynamically more susceptible to becoming diapirs. Likewise, three-dimensional geometry (*23*) would cause thinner cylindrical plumes (*24*), which are more likely to produce diapirs.

Phase transitions are not the only means for generating diapiric structures in the mantle. At higher *Ra*, between 10^7 and 10^8, diapirs (*18, 25*) are common in ordinary thermal convection. Phase transitions in the upper mantle can promote the generation of mantle diapirs at lower *Ra*, of order 10^6. This is a complicated phenomenon that warrants further investigation (*26*). These numerical simulations demonstrate that multiple phase transitions at the 670-km discontinuity can control the dynamics of plumes, in particular, by filtering out tepid deep-mantle plumes and preferentially concentrating hot plumes in the upper mantle. The diapiric flows (*27*), induced by phase transitions, may be important for understanding the episodic nature of hot-spot volcanism.

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27. The diapirs at the base of the lithosphere are several hundred kilometers wide, as one can estimate from the distance between the two arrows in Fig. 3 (270 km). For this grid resolution and the pixels used in the visualization, the width of the discernible conduit associated with the left plume in the bottom panel of Fig. 3 is about 30 km.

28. We thank D. Anderson, R. Boehler, and A. Chopelas for helpful discussions and continual encouragement. Research supported by the National Aeronautics and Space Administration and initiated at Maxwell's. S. Roe's help is gratefully acknowledged. We are grateful to IBM Corporation for processor time on the IBM-3090-600J computer.

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Regulation of B Cell Antigen Receptor Signal Transduction and Phosphorylation by CD45

LOUIS B. JUSTEMENT,* KERRY S. CAMPBELL, NADINE C. CHIEN, JOHN C. CAMBIER†

CD45 is a member of a family of membrane proteins that possess phosphotyrosine phosphatase activity, and is the source of much of the tyrosine phosphatase activity in lymphocytes. In view of its enzymatic activity and high copy number, it seems likely that CD45 functions in transmembrane signal transduction by lymphocyte receptors that are coupled to activation of tyrosine kinases. The B cell antigen receptor was found to transduce a Ca^{2+}-mobilizing signal only if cells expressed CD45. Also, both membrane immunoglobulin M (mIgM) and CD45 were lost from the surface of cells treated with antibody to CD45, suggesting a physical interaction between these proteins. Finally, CD45 dephosphorylated a complex of mIg-associated proteins that appears to function in signal transduction by the antigen receptor. These data indicate that CD45 occurs as a component of a complex of proteins associated with the antigen receptor, and that CD45 may regulate signal transduction by modulating the phosphorylation state of the antigen receptor subunits.

THE MEMBRANE GLYCOPROTEIN CD45 is a necessary participant in the regulation of signal transduction by a number of lymphocyte receptors. In both T and B cells, receptor-mediated generation of second messengers and changes in cellular activation can be modified by co–cross-linking of CD45 with these receptors, suggesting that CD45 may act as a common regulatory protein in lymphocytes (*1*). Direct evidence that CD45 is important for T cell activation has been provided by studies of CD4, CD8, and the T cell receptor:CD3 complex. CD45 regulates the phosphorylation state and activity of the tyrosine-specific kinase $p56^{lck}$, which is associated with CD4 and CD8, thereby affecting signal transduction via these receptors (*2*). Further, the ability of the T cell antigen receptor to transduce signals leading to second messenger generation and proliferation is dependent on CD45 expression (*3*). In contrast, direct evidence for participation of CD45 in signal transduction mediated by the B cell antigen receptor has not been reported.

The plasmacytoma cell line J558Lμm3, which expresses a transfected mIgM antigen receptor (*4*), does not mobilize Ca^{2+} in response to cross-linking of that receptor (*5*). However, agents that activate GTP (guanosine triphosphate)-binding proteins, such as aluminum fluoride, do mobilize Ca^{2+} in these cells. Thus, the receptor ap-

L. B. Justement, K. S. Campbell, N. C. Chien, J. C. Cambier, Division of Basic Sciences, Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine. J. C. Cambier, also at Department of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver, CO 80206

*Present address: Department of Microbiology, J-19, The University of Texas Medical Branch, Galveston, TX 77550.

†To whom correspondence should be addressed.

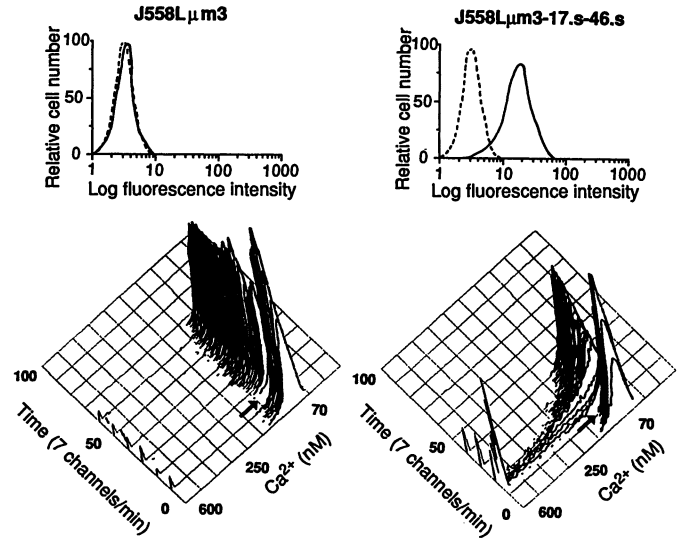

Fig. 1. Dependence of IgM-mediated signal transduction on CD45 expression. The top panels depict the amount of CD45 expressed by the mIgM positive parental plasmacytoma J558Lμm3 and the clone J558Lμm3-17.s-46.s, which was derived from the parent cell line after transfection with cDNA encoding CD45 (*11*). Shown are fluorescence histograms of cells that were exposed either to phycoerythrin-avidin alone (- - - -) or to biotinylated anti-CD45 (mAb) I3/2.5, and then to phycoerythrin-avidin (——) before analysis by flow cytometry. The bottom panels show the Ca^{2+}-mobilization response, as determined by flow cytometry (*5, 11*) which occurs after stimulation of either J558Lμm3 (left) or J558Lμm3-17.s-46.s (right) cells (10^6 cells/ml) with sheep antibody to mouse immunoglobulin at 25 μg/ml (SAMIg, added as indicated by the arrow).

pears to be uncoupled from second messenger generating systems at a point before activation of the operative GTP-binding protein. The mIgM on these cells is associated with accessory molecules (IgM-α, Ig-β, and Ig-γ), which were previously implicated in signal transduction by this receptor (*4, 6–9*). It was therefore possible that these cells lacked other proteins necessary for mIgM-mediated signal transduction. Analysis of the J558Lμm3 cell line by immunofluorescence with monoclonal antibody to CD45 (anti-CD45) (*10*) revealed that the cells fail to express CD45 on the cell surface (Fig. 1). To determine whether the failure of J558Lμm3 mIgM to induce mobilization of Ca^{2+} was due to a lack of CD45 expression, we transfected (*11*) a cDNA clone encoding the B220 isoform of CD45 into J558Lμm3 cells and assayed their ability to mobilize Ca^{2+} after mIgM cross-linking. The free Ca^{2+} content of transfected or untransfected cells was determined by flow cytometric analysis of cells treated with indo-1. The expression of CD45 did rescue mIgM-mediated signal transduction (Fig. 1). Analysis of a panel of clones that showed equivalent expression of mIgM on the cell surface but expressed varying amounts of CD45 on the cell surface revealed a direct correlation between CD45 expression and the concentration of intracellular Ca^{2+} attained in cells after cross-linking of mIgM (Fig. 2). These findings confirmed the demonstration that CD45 expression is essential for signal transduction via mIgM. Maximal Ca^{2+} mobilization responses were seen when CD45 expression reached ~15% of that found on mature, resting B cells or B cell lymphomas. It appears that the amount of CD45 present on mature B cells exceeds that needed for the function of the antigen receptor. On the basis of previous quantitation of mIgM (*12*) and CD45 (*13*), we estimate that the molar ratio of these proteins on the maximally responsive transfectants is 1:1 or greater, suggesting that these molecules may occur in a complex.

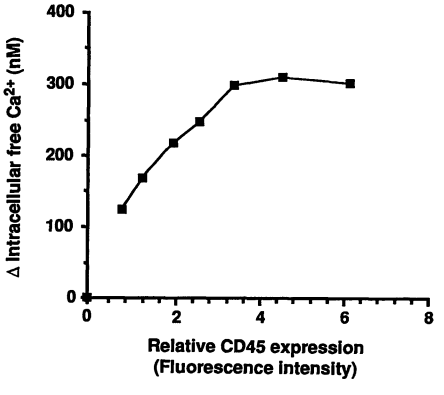

Fig. 2. Relation between CD45 expression and the ability of mIgM to transduce signals resulting in mobilization of Ca^{2+}. Clones of transfected cells were analyzed by indirect immunofluorescence to determine the amount of CD45 on the cell surface. Clones were selected based on graded expression of CD45 and equivalent expression of mIgM on their surface, and were analyzed to determine their relative ability to mobilize Ca^{2+} after mIgM cross-linking with 25 μg of SAMIg in a 1-ml sample of 10^6 cells. The expression of CD45 on eight selected clones was characterized by a single immunofluoresence peak. Thus, the relative change in CD45 expression was due to a change in CD45 expression by all cells in a clone, and was not due to the presence of one or more subpopulations expressing variable amounts of the protein. Relative CD45 expression is plotted as a function of the maximum change in the concentration of free intracellular Ca^{2+} ($[Ca^{2+}]_i$) achieved following stimulation with SAMIg. The maximum mean change in $[Ca^{2+}]_i$ after addition of antibody to mIg was determined as described (*5*). The data are from a single experiment that is representative of three experiments.

To determine whether cross-linking of CD45 on cells leads to loss of the antigen receptor from the cell surface, we incubated splenic B cells with anti-CD45 (RA3.3A1) for varied periods of time (*14*). Two-color immunofluorescence analysis was performed to assess modulation of CD45 and either mIgM or class I major histocompatibility complex (MHC) molecules. Cells were incubated in the absence or presence of anti-CD45 for 12 hours, after which surface receptor expression was measured by immunofluorescence (Fig. 3). Treatment (*15*) with anti-CD45 resulted in the loss of CD45 and mIgM from the surface of a proportion of cells in the population. These results may indicate that mIgM occurs as a complex with CD45. Class I MHC molecules (*16*) were not lost from the surface of B cells treated with anti-CD45, indicating that the effect is selective, if not specific.

It is likely that CD45 functions by dephosphorylating molecules at the inner face of the plasma membrane. The tyrosine phosphorylated subunits of the antigen receptor complex are potential substrates for CD45. In the cell membrane, mIgM is noncovalently associated with a complex of phosphoproteins consisting of three N-glycosylated polypeptides that occur as disulfide-linked heterodimers (*7, 8*). Membrane IgM and IgD associate with the subunits pp34 (Ig-γ) and pp37 (Ig-β) as well as with isotype-specific subunits pp32 (IgM-α) and pp33 (IgD-α). The α subunits are disulfide-linked with either β or γ subunits to form the heterodimers. These mIgM-associated proteins are phosphorylated on tyrosine residues in response to stimulation with AlF_4^- (*7*) or antibodies to mIg (*17*). Complexes of mIgM-associated proteins were immunoprecipitated from digitonin lysates of ^{32}P-labeled splenic B cells and mixed in vitro with CD45, which has been immunoprecipitated from the K46-17μmλ B cell lymphoma (*18*). This incubation mixture included NP-40 (0.6%) to release the α, β, and γ chains and make them accessible to CD45. The mIgM-associated proteins were rapidly dephosphorylated by CD45 in vitro (Fig. 4). A decrease in ^{32}P associated with all subunits was observed within 2 min and was maximal by 10 min. Control immunoprecipitates did not cause dephosphorylation. Dephosphorylation was blocked by addition of sodium orthovanadate (10 μM) or zinc chloride (1 mM), inhibitors of phosphotyrosine phosphatases (PTPase).

We next analyzed the ability of CD45 to modulate phosphorylation of the mIgM-associated protein complex in permeabilized cells treated with AlF_4^-. Co–cross-linking (*19*) of mIgM and CD45 with antibodies

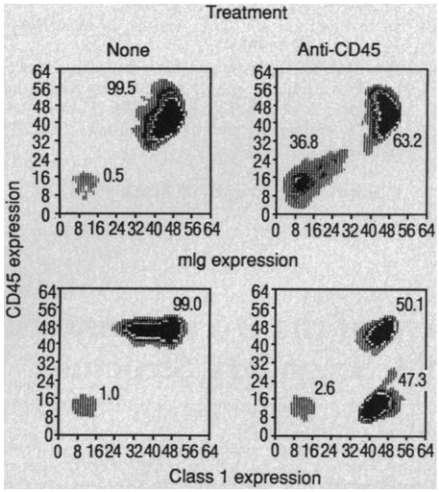

Fig. 3. Comodulation of mIgM with CD45. Resting splenic B cells were incubated in the absence (left panels) or presence (right panels) of anti-CD45 (RA3.3A1, 30 μg/ml) (*14*). No difference in either the viability or recovery of B cells was observed between untreated and anti-CD45 treated populations (90 and 89% recovery, respectively). Cells were subsequently stained (*14*) to determine the amount of CD45 and mIgM (top panels) or CD45 and class I (H2K) (bottom panels) on the cell surface based on two-color immunofluorescence. The amount of CD45 is shown on the *y* axis and the amount of mIgM or Class I is shown on the *x* axis. The percentage of total cells found in each subpopulation is indicated.

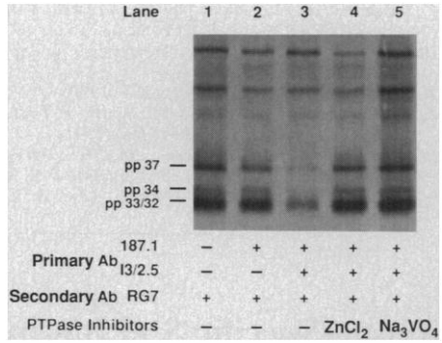

Fig. 5. Decreased labeling of the mIgM-associated phosphoproteins in intact splenic B cells after co–cross-linking of mIgM and CD45 (*19*). The amount of ^{32}P associated with the IgM-α (32 kD), -β (37 kD), and -γ (34 kD) subunits after AlF_4^- stimulation of intact cells was not affected by cross-linking of mIgM alone (lane 2). In contrast, co–cross-linking of mIgM and CD45 resulted in a significant decrease in ^{32}P associated with these subunits (lane 3), indicating that the close apposition of CD45 to mIgM may facilitate dephosphorylation of the mIgM-associated complex. Addition of phosphotyrosine phosphatase inhibitors (*24*), $ZnCl_2$ or Na_3VO_4 (1 mM and 10 μM, respectively), blocked the effect mediated by CD45 (lanes 4 and 5, respectively).

decreased the amount of ^{32}P associated with all subunits of the mIgM-associated protein complex (Fig. 5). This decrease was apparently not due to a loss of mIgM and its associated phosphoprotein complex to the sedimented cytoskeletal fraction because cross-linking of the antigen receptor alone did not cause a decrease in the phosphor-ylation of the α, β, and γ chains. Further, analysis of phosphoproteins extracted from cytoskeletal sedi-

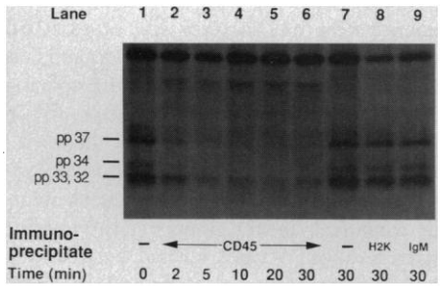

Fig. 4. Dephosphorylation of the mIgM-associated phosphoproteins mediated by CD45 in vitro (*18*). ^{32}P-labeled Ig-α, -β, and -γ subunits were incubated in vitro with CD45 (lanes 2 to 6) for 2 to 30 min. ^{32}P-labeled subunits were incubated for 30 min with irrelevant class I, or mIg immunoprecipitates to control for nonspecific dephosphorylation (lanes 8 and 9, respectively). Lane 1 shows the amount of ^{32}P associated with Ig-α, -β, and -γ subunit in immunoprecipitated but otherwise unmanipulated samples.

ments with buffers that destabilize the cytoskeleton did not reveal significant amounts of ^{32}P-labeled α, β, or γ chains, regardless of whether mIgM or CD45 (or both) had been initially cross-linked. Finally, in the presence of either sodium orthovanadate or zinc chloride, the decrease in ^{32}P-labeling of the mIgM-associated phosphoproteins (lanes 4 and 5) was inhibited, indicating that the observed decrease in ^{32}P-labeling reflects the activity of a tyrosine specific phosphatase. In T lymphocytes, co–cross-linking of CD45 with phosphotyrosine-containing proteins does not always lead to their dephosphorylation (*20*). Similarly, incubation of ^{32}P-labeled PLC-γl with CD45 in vitro does not result in dephosphorylation of tyrosine residues (*21*). Thus, CD45 apparently has restricted substrate specificity in vivo and in vitro. The fact that CD45 is capable of regulating the phosphorylation state of the mIgM-associated protein complex both in vivo and in vitro supports the hypothesis that interaction of these molecules may have important physiological consequences.

These findings suggest that CD45 is a component of a complex, which has been shown to include mIgM, the protein tyrosine kinase Lyn (*22*), and the mIgM-associated α, β, and γ subunits (*7, 8*). The ability of this complex to transduce signals may be subject to dynamic regulation by tyrosine phosphorylation (*23*) and dephosphorylation, with hyperphosphorylation leading to receptor inactivation. It is likely that CD45 plays a critical regulatory role in lymphoid cell activation after antigenic stimulation.

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10. The J558Lμm3 parental cell line was screened for CD45 message by mRNA dot blot analysis with the ^{32}P-labeled full-length B cell CD45 cDNA (B220 form) as a probe. By this method CD45-specific mRNA was detected in the parental cell line. However, no CD45 protein could be detected in the cell by protein immunoblotting or intracellular immunofluorescence staining. Immunofluorescence analysis of the parental cell line was carried out with the monoclonal antibody I3/2.5, which detects all CD45 isoforms, thus eliminating the possibility that differentiation of these cells was accompanied by a switch to the expression of a CD45 isoform other than the high molecular weight species, B220 [G. S. Jensen, S. Poppema, M. J. Mant, L. M. Pilarski, *Int. J. Immunol.* **1**, 229 (1990)].
11. The mIgM-positive, CD45-negative J558Lμm3 plasmacytoma was transfected with cDNA encoding the B220 isoform of CD45 [P. Johnson, L. Greenbaum, K. Bottomly, I. S. Trowbridge, *J. Exp. Med.* **169**, 1179 (1989)] by electroporation as described [W. F. Wade *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6297 (1989)]. Cells were selected in medium containing G418 (1 mg/ml). Drug resistant clones were analyzed for CD45 expression by immunofluorescence, and CD45-positive clones were isolated by repeated sorting and cloning. Briefly, 1×10^6 cells were washed in phosphate-buffered saline (PBS) containing 2% fetal bovine serum and 0.2% sodium azide (staining buffer) and incubated in the presence or absence (secondary control) of biotinylated I3/2.5 [I. S. Trowbridge, *J. Exp. Med.* **148**, 313 (1978)] for 30 min on ice. Cells were then washed three times in staining buffer and incubated for an additional 30 min on ice in the presence of phycoerythrin-avidin. Stained cells were analyzed on a Coulter Profile flow cytometer. For measurement of $[Ca^{2+}]_i$, cell lines were incubated with indo-1 AM (*5*). Briefly, cells (1×10^6) were incubated with indo-1 AM at a final concentration of 5 μM for 30 min at 37°C in Iscove's modified Dulbecco's medium (IMDM) containing 10 mM Hepes at pH 7.0. Cells were washed in IMDM/Hepes, pH 7.2, containing (5%) fetal calf serum and deoxyribonuclease (10 μg/ml) to remove unincorporated indo-1 AM. Flow cytometric analysis (1×10^6 cells per 1-ml sample) was carried out in an Ortho System 50H flow cytometer [L. B. Justement, J. C. Cambier, G. T. Rijkers, K. Fittschen, in *Noninvasive Techniques in Cell Biology*, S. Grinstein and J. K. Foskett, Eds. (Wiley-Liss, New York, 1990), pp. 353–374].
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14. Splenic B cells were isolated (*5*), and incubated in medium alone or in medium containing biotinylated anti-CD45 (RA3.3A1, 30 μg/ml) [R. L. Coffman and I. L. Weissman, *Nature* **289**, 681 (1981)] at a concentration of 1×10^6 cells/ml for 12 hours at

37°C. After the initial incubation, cells were washed twice in staining buffer and incubated for 20 min at 4°C in the presence of biotinylated RA3.3A1. Cells were washed twice in staining buffer and then incubated a second time at 4°C in the presence of phycoerythrin-avidin and either FITC-labeled anti-Ig (187.1, rat monoclonal antibody to mouse κ light chain) or antibody to class I (M1/42.398, rat monoclonal antibody to mouse H2K). Cells were washed twice with staining buffer and analyzed by two-color flow cytometry.

15. Reciprocal loss of CD45 from the cell surface after treatment of B cells with anti-Ig was not detected. Cells express ten times more CD45 molecules on their surface than mIgM (*12, 13*). Thus, if each mIgM interacts with one CD45 molecule, loss of every mIg molecule from the surface of the cell would result in, at most, a 10% decrease in the number of CD45 molecules on the cell. Such a decrease would not be detected in this assay.
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18. Splenic B cells were incubated in phosphate-free medium for 1 hour at 37°C, washed once, and permeabilized with α-lysophosphatidylcholine (7) [A. R. Mire, R. G. Wickremasinghe, R. Michalevecz, A. V. Hoffbrund, *Biochim. Biophys. Acta.* **847**, 159 (1985)]. [γ^{32}P]ATP (150 μCi) was added to 6×10^7 cells and AlF_4^- (10 μM $AlCl_2$ and 30 mM NaF) was then added to stimulate phosphorylation of the mIgM-associated protein complex. For each reaction, 2×10^7 cells were lysed in buffer containing 1% digitonin as well as protease and PTPase inhibitors; ^{32}P-labeled phosphoproteins (pp37, −34, −33, and −32) were coimmunoprecipitated with mIg by means of monoclonal antibody (MAb) 187.1 (*14*), as described (7), with the exception that in the last two washings, PTPase inhibitors were omitted. Other membrane proteins or CD45 were isolated from the K46-17μmλ B cell lymphoma. These cells were washed twice in PBS and lysed in buffer containing protease inhibitors (7), 1% NP-40, 0.15 M NaCl, and 0.01 M sodium phosphate, pH 7.2, for 1 hour on ice. The lysates were centrifuged at 12,000*g* for 20 min at 4°C. For each reaction, the supernatant from 5×10^7 cells was incubated with the MAb I3/2.5 (rat anti-CD45) (10 μg/ml) or with either of the control MAbs (187.1 or M1/42.398) (10 μg/ml) at 4°C for 1 hour. Protein-G beads (Pharmacia) were added, and the samples were mixed for an additional hour at 4°C. For the reaction in lane 7, only Protein-G beads were added to the K46-17μmλ lysate. Beads were washed six times in sodium phosphate buffer containing NP-40 (0.2%). To initiate the dephosphorylation reaction, beads bound with ^{32}P-labeled subunits were mixed with beads bound to either CD45, mIgM, or H2K in a final volume of 50 μl. The reaction buffer [N. K. Tonks, C. D. Diltz, E. H. Fischer, *J. Biol. Chem.* **263**, 6722 (1988)] contained 25 mM imidazole-HCl, pH 7.2, 0.15 M NaCl, β-mercaptoethanol (0.1%), and NP-40 (0.6%), which causes dissociation of the α, β, and γ subunits from mIgM (7). Mixtures were incubated at 37°C for varied periods of time and the reaction was stopped by addition of 2× SDS–polyacrylamide gel electrophoresis (PAGE) sample buffer followed by boiling for 5 min. Phosphoproteins were analyzed by SDS-PAGE (10% gel) followed by autoradiography.
19. Splenic B cells (2×10^7), were phosphate-depleted (7) and incubated with saturating concentrations of antibodies (187.1, or I3/2.5, or both) for 10 min on ice. After the addition of primary antibodies, cells were washed three times in ice-cold phosphate-free medium and the secondary cross-linking mAb, RG7 (mouse antibody to rat κ light chain), was added. Cells were incubated for an additional 10 min on ice and washed once. The cells were then permeabilized and mixed with [γ^{32}P]ATP and AlF_4^- (*7, 18*). The cells were incubated for 10 min at 37°C and lysed in buffer containing 1% digitonin. ^{32}P-labeled subunits were immunoprecipitated with Protein-G beads after the addition of 187.1 (10 μg/ml) (*18*). The mIgM-associated phosphoprotein complex was visualized by autoradiography after separation by SDS-PAGE (10% gel) under reducing conditions.
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Tissue-Specific Splicing in Vivo of the β-Tropomyosin Gene: Dependence on an RNA Secondary Structure

DOMENICO LIBRI, ANNA PISERI, MARC Y. FISZMAN

The β-tropomyosin gene in chicken contains two mutually exclusive exons (exons 6A and 6B) which are used by the splicing apparatus in myogenic cells, respectively, before (myoblast stage) and after (myotube stage) differentiation. The myoblast splicing pattern is shown to depend on multiple sequence elements that are located in the upstream intron and in the exon 6B and that exert a negative control over exon 6B splicing. This regulation of splicing is due, at least in part, to a secondary structure of the primary transcript, which limits in vivo the accessibility of exon 6B in myoblasts.

IN MOST HIGHER EUKARYOTES GENES the coding region is interrupted by noncoding sequence segments (intervening sequences or introns) which are excised from the full copy transcript of the gene (pre-mRNA) during the splicing process. Variable use of the coding regions (exons) of the same gene to obtain partially different mRNA's is the widespread process known as alternative splicing.

The β-tropomyosin gene in chicken codes for three related isoforms of tropomyosin and contains a pair of mutually exclusive exons (exons 6A and 6B) that are expressed, repectively, in smooth muscle cells or nonmuscle cells (exon 6A) or in skeletal muscle (exon 6B). Myogenic cells in culture express exon 6A before differentiation and exon 6B after differentiation (myoblast and myotube stage, respectively).

On the basis of computer predictions (*1, 2*) or mutagenesis studies in vivo (*3*) and in vitro (*4*), we have proposed that a secondary structure could be one of the cis-regulating elements that controls alternative splicing of this gene.

The potential influence of secondary structures on alternative splicing has been suggested on the basis of in vitro and in vivo splicing of mRNA precursors containing inverted repeat sequences that flank exons or splice sites (*5, 6*). A threshold value exists in vivo (but not in vitro) for the distance between the two inverted repeats (that is the length of the hairpin loop), which has been related to the existence of a competition between the interaction of the primary transcript with heterogeneous nuclear, spliceosomal ribonucleoproteins (RNP's) or both, and the folding of the newly transcribed region into a secondary structure (*6*). It has been proposed (*6*) that a "window" of about 100 nucleotides (nt) exists behind the transcribing polymerase within which the primary transcript is "naked" and free to fold. However, a naturally occurring example of a secondary structure, which influences alternative splicing in vivo has not yet been reported. Two cases have been described (*7, 8*) in which secondary structures can affect splice site selection in vitro, but these results have not been supported by parallel in vivo splicing studies.

We have earlier shown (*3*) that the sequence near the acceptor site of exon 6B contains at least two, nonoverlapping, cis elements that are part of a negative splicing control preventing the use of exon 6B in myoblasts. Mutation of either of them activates splicing of the exon even in myoblasts or nonmyogenic cells. One of these elements is contained in the long polypyrimidine stretch (about 90 bp) located between the branchpoint (position −105) and the terminal AG of the intron, and the other is contained in the exon itself. Earlier we proposed a model whereby a secondary structure of the primary transcript would be responsible in myoblasts for the skipping of exon 6B, while exon 6A would be spliced as a default choice. Furthermore, a specific factor would be expressed in myotubes, which would disrupt the structure (or pre-

D. Libri and M. Y. Fiszman, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France.
A. Piseri, Dipartimento di Genetica e Biologia dei Microorganismi, via Celoria, 26, 20131 Milano, Italy.