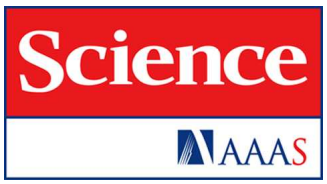

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Activation of Auditory Cortex During Silent Lipreading

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Watching a speaker's lips during face-to-face conversation (lipreading) markedly improves speech perception, particularly in noisy conditions. With functional magnetic resonance imaging it was found that these linguistic visual cues are sufficient to activate auditory cortex in normal hearing individuals in the absence of auditory speech sounds. Two further experiments suggest that these auditory cortical areas are not engaged when an individual is viewing nonlinguistic facial movements but appear to be activated by silent meaningless speechlike movements (pseudospeech). This supports psycholinguistic evidence that seen speech influences the perception of heard speech at a prelexical stage.

During face-to-face conversation, the perception of speech is reliably improved by watching the speaker's lips moving (lipreading) as the words are spoken (*1*), particularly in noisy surroundings (*2*). The influence of these visual cues on auditory speech perception is usually outside the observer's awareness but becomes apparent when they are not synchronous with heard speech. This is experienced, for example, when watching a poorly dubbed movie, and is evidenced experimentally by the McGurk effect when an auditory percept is modified by lipreading (*3*).

Although research with positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) has refined the cerebral localization of auditory speech perception (*4*), the regions involved in the visual perception of articulatory movements from a speaker's face have not yet been precisely identified. How information from these distinct modalities is integrated to produce coherent and unified perception of speech during ordinary face-to-face conversation is an important question. The level at which these visual cues exert an influence on auditory speech perception is uncertain, but psychophysical evidence suggests that audiovisual integration of linguistic signals occurs before the stage of word identification, referred to as the prelexical level, and possibly at the stage of phonetic categorization (*5*).

In fMRI studies of normal hearing individuals we compared cerebral regions activated in silent lipreading with those activated during heard speech in the absence of visual cues to find out whether there is a common pathway by which information in visual and auditory modalities is integrated during face-to-face conversation. In two further experiments, we manipulated the linguistic specificity of these visual cues to explore at what stage dynamic facial gestures might influence auditory speech perception. For all experiments we used a design in which contrasting 30-s epochs of experimental (ON) and baseline (OFF) conditions were alternated over a total scanning time of 5 min (*6*). Differential activation between ON and OFF periods was estimated by subsequent analysis (*7*).

In experiment 1 the localization of brain areas involved in auditory speech perception was confirmed in five right-handed volunteers. During the ON condition, participants listened to spoken words presented through headphones and were asked to repeat silently to themselves each word as it was heard (*8*). During the OFF condition, there was no auditory stimulation, but participants were instructed to rehearse silently the number "one" at 2-s intervals—the same rate at which the words were presented aloud in the ON condition. These instructions were intended both to focus participants' attention on the stimuli in the ON condition and to activate cortical regions involved in internally generated speech consistently during both conditions. The comparison of these two conditions (Table 1) yielded bilateral activation of Brodmann areas (BA) 41, 42, and 22, pre-

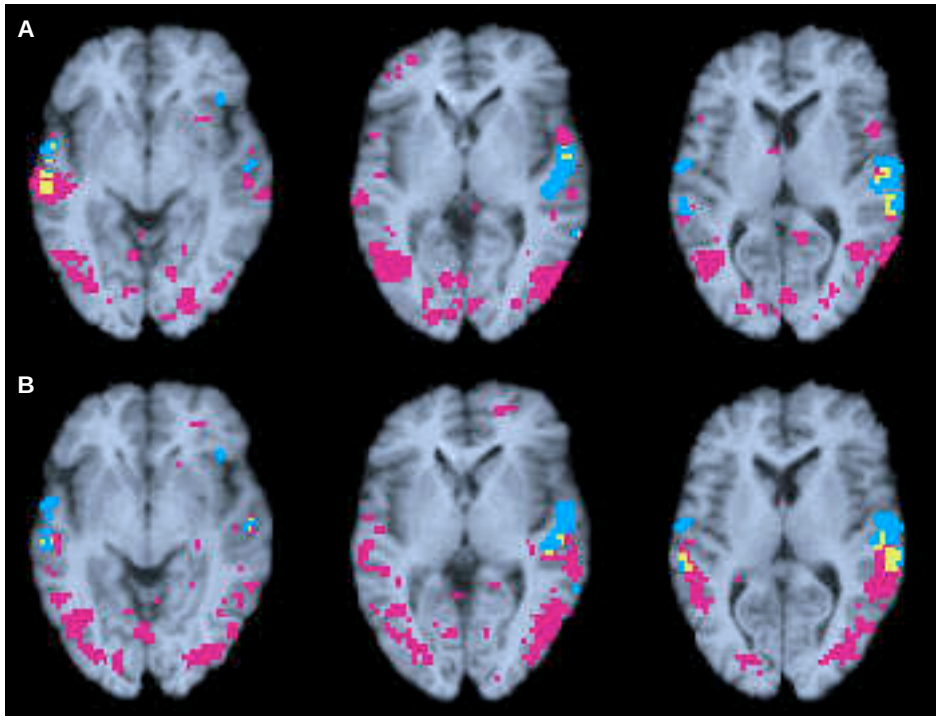

Fig. 1. Voxels colored purple indicate brain areas activated by silent lipreading in experiment 2 (**A**) and its replication (**B**) overlaid on areas activated during auditory speech perception in experiment 1 (blue voxels). Yellow voxels indicate regions activated in common by silent lipreading and heard speech. These generic brain activation maps are superimposed on spoiled GRASS MR images centered at 1 mm (left), 6.5 mm (center), and 12 mm (right) above the intercommissural (AC-PC) line. The left side of each image corresponds to the right side of the brain.

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viously shown to be involved in auditory speech perception (*4*). Activation in these auditory regions was more extensive in the left hemisphere, consistent with its dominant role in language processing.

Experiment 2 was designed to identify in the same five individuals the brain regions activated during silent lipreading. In the ON (lipreading) condition, participants watched a videotape of a face silently mouthing numbers at a rate of one number every 2 s and were instructed to repeat silently the numbers they saw being mouthed (*9*). In the OFF condition, participants viewed a static face and were asked to repeat silently to themselves the number "one" at 2-s intervals. The following brain regions demonstrated a significant signal increase bilaterally during the ON (lipreading) condition: extrastriate cortex (BA 19), inferoposterior temporal lobe (BA 37), angular gyrus (BA 39), and of specific interest, superior temporal gyri including BA 41, 42, and 22 (primary auditory and auditory association cortices, respectively) (Fig. 1 and Table 1).

These areas may subserve the component processes activated during silent lipreading. The extrastriate cortex and inferoposterior temporal lobe (which includes area V5) have been implicated in the detection of coherent visual movement (*10*), and activation of this region can be related to the contrast between viewing moving and still lips in the two conditions. The angular gyrus is involved in the mapping of visually presented inputs (including words and numbers) to the appropriate linguistic representations (*11*), and in this experiment, it may be involved in mapping facial speech cues to their appropriate verbal representation. The most intriguing finding was the activation of lateral temporal auditory cortex during silent lipreading. These areas overlapped considerably with those active during auditory speech processing (*4*) in these same individuals during experiment 1. However, in experiment 2 there was no auditory input other than the background scanner noise, which was constant in both conditions. The neural substrate common to heard and seen speech is illustrated in Fig. 1A.

This result provides a possible physiological basis for the enhancing effects of visual cues on auditory speech perception and the McGurk illusion (*12*). Furthermore, activation of primary auditory cortex during lipreading suggests that these visual cues may influence the perception of heard speech before speech sounds are categorized in auditory association cortex into distinct phonemes (*13*). The direct activation of auditory cortex by information from another modality may, in this instance, be a consequence of the early development of a crossmodal process because, especially for infants, heard speech is usually accompanied by the sight of the speaker (*14*).

To further examine the components of the response to silent lipreading, we manipulated the stimuli in the OFF (baseline) condition to engage initially the detection of lip movements per se (experiment 3) and then the perception of lip and mouth movements that resemble real speech (experiment 4) (Table 2). In both experiments the ON condition involved lipreading and silent repetition of the mouthed numbers. Five new participants were recruited for this study. These individuals also completed a refined version of experiment 2 intended to replicate our original finding of auditory cortical activation during silent lipreading (*15*) (Fig. 1B).

In experiment 3, participants were presented during the OFF condition with examples of facial gurning (consisting of bilateral closed-mouth gestures or twitches of the lower face) produced at the same rate as the mouthed numbers in the ON condition. They were asked to attend closely to the stimuli and to count silently the number of facial gestures they saw. This contrast was designed to investigate whether activation of temporal cortex during silent lipreading might simply be a consequence of visually perceiving motion from the lower face. However, the persistence of differential activation of temporal cortex bilaterally during the ON (lipreading) condition suggests

Table 1. Major regional foci of differential activation (*23*). FPQ, fundamental power quotient.

Coordinates (mm)			Cluster size	Max (FPQ)	Total (FPQ)	Side	Cerebral region	BA	Active condition
x	*y*	*z*							
Experiment 1: Heard speech (ON) versus no auditory stimulus (OFF)									
−49	−19	13	45	4.8	142	L	Transverse temporal gyrus	41	ON
−49	−14	6	32	5.2	104	L	Insula	–	ON
61	−11	13	11	3.7	30	R	Superior temporal gyrus	42	ON
61	−33	13	10	3.4	27	R	Superior temporal gyrus	22	ON
−55	−8	3	5	3.0	13	L	Superior temporal gyrus	22	ON
Experiment 2: Lips mouthing numbers (ON) versus still lips (OFF)									
−49	−64	13	67	10.4	283	L	Angular gyrus	39	ON
61	−17	3	52	5.7	186	R	Superior temporal gyrus	22	ON
46	−64	13	47	7.2	179	R	Angular gyrus	39	ON
52	−58	8	34	5.0	113	R	Inferoposterior temporal lobe	37	ON
−40	−78	8	26	4.2	76	L	Middle occipital gyrus	19	ON
55	−25	8	19	5.3	59	R	Superior temporal gyrus	42	ON
−52	−19	13	15	3.3	40	L	Transverse temporal gyrus	41	ON
−52	−22	8	10	2.9	25	L	Superior temporal gyrus	42	ON
−61	−28	3	10	3.1	26	L	Superior temporal gyrus	22	ON
26	−83	17	9	2.8	22	R	Middle occipital gyrus	19	ON
Experiment 3: Lips mouthing numbers (ON) versus gurning (OFF)									
61	−22	13	13	3.2	37	R	Superior temporal gyrus	22	ON
−58	−28	3	11	3.1	29	L	Superior temporal gyrus	22	ON
49	−50	17	8	2.7	20	R	Angular gyrus	39	ON
−55	−53	8	6	3.1	16	L	Inferoposterior temporal lobe	37	ON
3	56	8	40	3.9	111	R	Frontal pole	10	OFF
0	47	−1	25	4.4	77	R	Medial frontal lobe	32	OFF
−6	−47	8	15	3.9	44	L	Posterior cingulate gyrus	30	OFF
3	−50	22	11	3.1	28	R	Posterior cingulate gyrus	30	OFF
Experiment 4: Lips mouthing numbers (ON) versus lips mouthing pseudospeech (OFF)									
26	3	−10	14	3.6	38	R	Amgydala	–	OFF
−32	19	−6	13	3.6	35	L	Insula	–	OFF
40	14	−1	7	2.7	17	R	Insula	–	OFF

Table 2. Experimental design for experiments 2 through 4.

Linguistic processes	Processes engaged during the ON condition	Processes engaged during the OFF condition		
	All experiments	Expt. 2*	Expt. 3	Expt. 4
Lexical mouth movements	+	−	−	−
Prelexical mouth movements	+	−	−	+
None (movement only)	+	−	+	+

*In experiment 2, participants viewed a static lower face during the OFF condition.

that the complex lower facial movements present in the OFF condition do not activate the auditory sites involved in silent lipreading. Bilateral activation of posterior cingulate cortex (BA 30) and the medial frontal lobe and frontal pole (BA 32 and 10) was observed during the OFF condition (facial gurning). These regions have been implicated in attention-demanding tasks (*16*) and may relate to the unfamiliar nature of gurning stimuli by comparison with familiar facial speech movements.

The aim of experiment 4 was to determine whether auditory cortex could be activated by visual perception of lip movements that were phonologically plausible (visible pseudospeech) but did not form coherent words (*17*). In the OFF condition, participants again counted silently the number of pseudospeech movements they saw. Under these conditions there was no net superior temporal activation, suggesting that visible pseudospeech may engage similar cortical regions to those used in normal lipreading. This finding supports the suggestion that linguistic facial gestures influence heard speech at a prelexical level. Bilateral activation of the insula (left > right) was detected during pseudospeech, which might be expected by the increased demand placed on phonological processing in the absence of semantic context, and is consistent with a role for the insula in articulatory processing (*18*). Activation in the amygdala probably relates to the heightened emotional salience of open- as opposed to close-mouthed facial expressions (*19*) or expressive movements in general (*20*).

In summary, these experiments suggest that silent lipreading activates auditory cortical sites also engaged during the perception of heard speech. In addition, it appears that auditory cortex may be similarly activated by visible pseudospeech but not by nonlinguistic closed-mouth movements. This adds physiological support to the psychological evidence that lipreading modulates the perception of auditory speech at a prelexical level (*5*, *21*) and most likely at the stage of phonetic classification.

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6. The brain regions reported active in each experiment were those demonstrating periodic signal change, in phase either with the ON or OFF condition. The OFF condition was always presented first. There was no auditory stimulation in experiments 2 to 4 except for the scanner noise. As this was the same during all ON and OFF conditions, any signal change related to the background noise would not be periodic and would therefore not contribute to the estimated experimental effect. For all experiments reported, all participants gave informed and written consent.
7. Gradient echo echoplanar MRI data were acquired with a 1.5-T GE Signa system retrofitted with Advanced NMR operating console with a quadrature, birdcage head coil. One-hundred T_2^*-weighted images depicting BOLD contrast [K. K. Kwong *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 5675 (1992)] were acquired with an in-plane resolution of 3 mm (TR = 3 s; TE = 40 ms) at each of 10 near-axial noncontiguous 5-mm-thick slices (with 0.5-mm interslice gap) positioned parallel to the AC-PC line to include the visual, auditory, and inferior frontal cortices. After movement correction by realignment and regression of each realigned time series on the vector of estimated translations and rotations in three dimensions [K. J. Friston, S. C. R. Williams, R. J. Howard, R. S. J. Frackowiak, R. Turner, *Magn. Reson. Med.* **35**, 346 (1996)], a sinusoidal regression model was fitted by pseudogeneralized least squares to the fMRI time series observed at each voxel (*22*). The model included a pair of sine and cosine terms, with coefficients (γ, δ), at the fundamental frequency of alternation between experimental conditions. The sum of squared coefficients ($\gamma^2 + \delta^2$) provided an estimate of the power in the signal at the fundamental frequency, which was divided by its standard error to estimate the fundamental power quotient (FPQ). The sign of γ indicated the timing of a signal intensity increase relative to the experimental input function: positive indicated increased signal intensity during the first (OFF) condition; negative indicated increased signal intensity during the second (ON) condition [E. T. Bullmore *et al.*, *NeuroImage* **4**, 16 (1996)]. A nonparametric approach was used to assess the significance of observed values of FPQ with minimal assumptions about the distribution of FPQ under the null hypothesis [(*22*); M. J. Brammer, S. C. R. Williams, E. T. Bullmore, *NeuroImage* **3**, 52 (1996)]. Each observed fMRI time series was randomly permuted 10 times and FPQ estimated for each randomized time series exactly as for the observed data. Maps of estimated FPQ at each voxel of the observed images were then registered in a standard coordinate space [J. Talairach and P. Tournoux, *A Coplanar Stereotactic Atlas of the Human Brain* (Thieme Verlag, Stuttgart, Germany, 1988)] and smoothed by a Gaussian filter (full width at half maximum 7 mm) to accommodate individual variability in gyral anatomy. Maps of FPQ estimated in the randomized images were smoothed by an identical filter. Finally, the median FPQ estimate over all participants at each voxel of the observed images was tested against a null distribution of median FPQ ascertained from the randomized images. If the observed median FPQ exceeded the critical value for a test of size 5×10^{-4}, that voxel was considered activated and represented in color on the gray scale background of a coregistered (spoiled GRASS) MR image. By significance testing at this level, false-positive activation should account for less than 10 colored voxels over the whole generic brain activation map.
8. The stimuli in experiments 1 and 2 comprised spoken or mouthed numbers ranging from 1 to 10, uttered in random order at 0.5 Hz. The stimuli were selected to be equally comprehensive when presented in either the auditory or visual domain. All participants were shown examples of the stimuli before the scanning session and were able to lipread the numbers. In experiment 1 no visual stimulus was present during either condition.
9. Throughout the experiment the participant's field of view was restricted to the lower half of the face to minimize the influence of direction of gaze and facial identity processing. The same face was used for both moving- and static-face stimuli.
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12. In a further experiment with the same individuals reported in experiments 1 and 2, we contrasted hearing speech with concordant lip movements (face-to-face conversation) with the same lip movements in the absence of sound. This comparison resulted in marked attenuation of the expected signal from auditory cortical sites in the first condition, presumably because they were canceled out by activation of these areas during the second condition (silent lipreading). This concurs with our conclusion that silent lipreading stimulates those auditory cortical sites activated when listening to speech.
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15. To address the possibility that activation of auditory cortex during silent lipreading in experiment 2 was due to greater articulatory demands in this ON condition (repeating back numbers), we repeated the experiment but modified the task instructions in the OFF condition by asking participants to count silently from one, rather than simply repeating "one," to match more closely the articulatory demands of the ON (lipreading) condition. This comparison again demonstrated activation of auditory cortical areas in response to lipreading (Fig. 1B), as shown initially in experiment 2. The largest cluster of (*13*) voxels in temporal cortex that were coincidentally activated by auditory speech perception (experiment 1), and by silent lipreading in this replication of experiment 2, had a diameter of 9 mm and was centered at Talairach coordinates $x = -56$, $y = -26$, $z = 9.5$. This center point lies in BA 42 and the cluster extends superiorly into BA 41 and posteriorly and inferiorly into BA 22, thus replicating the findings of experiment 2. In addition, our previous PET study of silent articulation reveals prominent activation of the inferior frontal gyrus but only minimal activation of temporal cortex and no activation in BA 41 during internal rehearsal [P. K. McGuire *et al.*, *Psychol. Med.* **26**, 29 (1996)]. Hence, if in our initial study the temporal cortical activation had been due to increased articulation, we would have also expected to see substantial differential activation in the inferior frontal gyrus during the lipreading condition. In fact there were only a few voxels activated in this region in experiment 2, suggesting that the two conditions in this experiment did not differ importantly in terms of their articulatory demands.
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17. The stimuli comprised a videotape of the same speaker mouthing a random string of syllables using a wide range of phonological structures: vowels included a range of non-English diphthongs (such as the German "ü"); consonants included some combinations that are not common in English (such as "pf-," "fth-," and "plm-"). We have classified these as examples of pseudospeech that were perceived as phonetically plausible but were not recognizable as words.
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23. Areas of activation shown are those corresponding to the largest clusters. A complete list of activations can be obtained from the corresponding author. Within each cluster the coordinates of the voxel with the maximum FPQ are shown.
24. Supported by an Oxford University Pump Priming grant and the Bethlem and Maudsley Research Fund. E.T.B. and P.K.M. were supported by the Wellcome Trust, G.A.C. by the Medical Research Council of Great Britain, and P.W.R.W. by British Telecom. We are grateful to I. C. Wright and R. Howard and to two anonymous reviewers for their helpful comments on this manuscript.

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Repression of c-*myc* Transcription by Blimp-1, an Inducer of Terminal B Cell Differentiation

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Transcription of c-*myc* in plasma cells, which are terminally differentiated B cells, is repressed by plasmacytoma repressor factor. This factor was identified as Blimp-1, known for its ability to induce B cell differentiation. Blimp-1 repressed c-*myc* promoter activity in a binding site–dependent manner. Treatment of BCL_1 lymphoma cells with interleukin-2 (IL-2) plus IL-5 induced Blimp-1 and caused a subsequent decline in c-Myc protein. Ectopic expression of Blimp-1 in Abelson-transformed precursor B cells repressed endogenous c-Myc and caused apoptosis; Blimp-1–induced death was partially overcome by ectopic expression of c-Myc. Thus, repression of c-*myc* is a component of the Blimp-1 program of terminal B cell differentiation.

C-Myc functions at a critical decision point of cell growth to favor proliferation and to block terminal differentiation (*1*). c-Myc is present in dividing cells but is not expressed in quiescent or terminally differentiated cells (*2*); addition of exogenous c-Myc blocks terminal differentiation of several hematopoietic cell lines (*3*) and of myogenic cells (*4*), whereas inhibitors of c-Myc expression accelerate terminal differentiation of promonocytic HL60 cells (*5*), M1 leukemic myeloid cells (*6*), F9 teratocarcinoma cells (*7*), and human esophageal cancer cells (*8*).

Murine plasmacytomas are the transformed counterparts of plasma cells, which are terminally differentiated, nondividing B lymphocytes (*9*). Plasmacytomas have a characteristic reciprocal chromosomal translocation that juxtaposes one allele of the c-*myc* gene with an immunoglobulin heavy- or light-chain locus (*10*). The translocated c-*myc* allele is deregulated and overexpressed; however, the nontranslocated c-*myc* allele is transcriptionally silent (*1*). This state is thought to correspond to the silent state of the c-*myc* gene in normal plasma cells.

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A plasmacytoma-specific protein, plasmacytoma repressor factor (PRF), binds in the c-*myc* promoter 290 base pairs (bp) 5′ of the P1 transcriptional start site. PRF represses c-*myc* transcription in plasmacytomas and has not yet been cloned (*11*). The PRF binding site is similar in sequence to the interferon-stimulated response elements (ISREs) in many interferon-regulated genes (*12*) and to the positive regulatory domain 1 (PRD1) sequence of the human interferon-β (IFN-β) gene (*13*). Electrophoretic mobility shifts assays (EMSAs) with nuclear extracts from the plasmacytoma P3X63-Ag8 (P3X) and a c-*myc* promoter probe containing the PRF site confirmed that both ISRE and PRD1 oligonucleotides could compete for binding of PRF in this assay; PRD1 oligonucleotides competed more strongly than ISRE oligonucleotides (*14*).

PRD1-BF1 is a human zinc finger protein that was cloned by virtue of its ability to bind to the PRD1 site; PRD1-BF1 inhibits transcription of the IFN-β promoter (*13*). Recently the murine homolog of PRD1-BF1, Blimp-1, was identified as a protein that is induced upon stimulation of the BCL_1 B cell lymphoma line with interleukin-2 (IL-2) plus IL-5 (*15*). Ectopic expression of Blimp-1 can drive B cell terminal differentiation, and Blimp-1 is expressed only in plasmacytomas and mature B cells; however, its mechanism of action is not well understood (*15*). On the basis of cross-competition of their binding sites, common transcriptional repressor activity, and plasmacytoma-specific expression, we hypothesized that PRF might be identical to Blimp-1.

To test this hypothesis, we transfected 293T human kidney fibroblast cells with an expression plasmid encoding Blimp-1. An immunoblot developed with antiserum to Blimp-1 revealed that Blimp-1 was present in nuclear extracts from P3X plasmacytomas and in the transfected 293T cells but not in 18-81 precursor B cells (pre-B cells) or in mock-transfected 293T cells (Fig. 1). EMSAs were then done with these extracts with an oligonucleotide probe corresponding to the c-*myc* PRF site (Fig. 1). Complexes of identical mobility were observed for endogenous PRF in P3X cells and for the Blimp-1–transfected 293T cells, whereas no complex was detected for 18-81 or mock-transfected 293T cell extracts. The sequence specificity of these complexes was shown by the ability of PRF but not an unrelated sequence to compete the complexes. Finally, the complex from P3X extracts was completely ablated by antiserum against Blimp-1 but not by naïve antiserum. Thus, the protein in P3X cells that we identified as PRF is immunologically related to Blimp-1. The results obtained with EMSA and antibody ablation provide evidence that the c-*myc* repressor PRF is encoded by the *blimp-1* gene.

A site-directed mutation in the c-*myc* PRF site increases promoter activity 30-fold in plasmacytomas, which express PRF, but has no effect in fibroblasts and pre-B cells, which do not express the protein, showing that PRF represses c-*myc* transcription (*11*). To investigate the functional relation be-

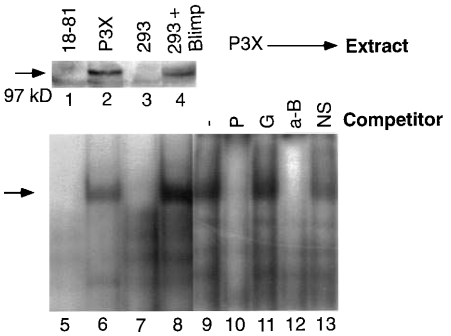

Fig. 1. Blimp-1 binds to the c-*myc* PRF site. (Top) Nuclear extracts from various cells were prepared as described (*26*) and subjected to immunoblot with antibody to Blimp-1; arrow indicates Blimp-1. Lane 1, 18-81 cells; lane 2, P3X cells; lane 3, mock-transfected 293T cells; lane 4, Blimp-1–transfected 293T cells. (Bottom) Lanes 5 to 8, the same extracts were used for EMSA with a 25-bp PRF oligonucleotide (*26*). Lanes 9 to 13, EMSA of P3X nuclear extracts with PRF oligonucleotide probe in the presence of no competitor (lane 9), PRF oligo tetramer (lane 10), GATA (nonspecific) tetramer (lane 11), rabbit antiserum to Blimp-1 (lane 12), and naïve rabbit serum (lane 13). Arrow indicates the protein-DNA complexes.