Localization of the 47 kDa phosphoprotein involved in the respiratory-burst NADPH oxidase of phagocytic cells

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A 47 kDa phosphoprotein is involved in the respiratory-burst oxidase of phagocytic cells. After stimulation of neutrophils with phorbol myristate acetate, this phosphoprotein was identified in both the cytosol and membranes. Peptide mapping of the two forms resulted in identical patterns of phosphopeptides. Dose-response curves for accumulation of phosphoprotein in the two sites were very similar, whereas the detection of the phosphoprotein in the cytosol preceded that in the membranes. The membrane-associated 47 kDa phosphoprotein was absent from the neutrophils of patients with X-chromosome-linked chronic granulomatous disease, which lack cytochrome b_{-245} , and intermediate levels were detected in the membranes of their heterozygote carrier mothers. Activation of the neutrophil oxidase system appears to be dependent upon phosphorylation of the cytosolic 47 kDa protein and its association with cytochrome b_{-245} in the membranes. It is probably the cytosolic factor required for reconstitution of the active oxidase in cell-free systems.

INTRODUCTION

There is now a great deal of evidence that the reversible phosphorylation of proteins is involved in the activation and deactivation of the superoxide-generating respiratory burst of phagocytic cells (Schneider *et al.*, 1981; White *et al.*, 1984; Segal *et al.*, 1985; Okamura *et al.*, 1988). This membrane-associated, NADPH-dependent, electron-transport system has an important role in the killing and digestion of microbes (Selvaraj & Sbarra, 1966). In normal neutrophils activation of the respiratory burst by a variety of soluble stimuli, such as chemotactic peptides and phorbol esters, and opsonized particles, is accompanied by the enhanced phosphorylation of a number of proteins (Schneider *et al.*, 1981; Andrews & Babior, 1983). These include a 47 kDa protein that appears to be directly involved in the activation process (Segal *et al.*, 1985; Hayakawa *et al.*, 1986; Heyworth & Segal, 1986), and both the 23 and 90 kDa subunits of cytochrome b_{-245} , the terminal electron transporter of the chain (Garcia & Segal, 1988).

Chronic granulomatous disease (CGD) is an inherited syndrome characterized by a greatly increased susceptibility to bacterial and fungal infections. It results from the failure of the patient's phagocytes to generate a respiratory burst and produce oxygen radicals (Holmes *et al.*, 1967). There are two main patterns of inheritance. The genetic lesion in the commonest, X-chromosome-linked form is located in the gene coding for the 90 kDa membrane glycoprotein component of cytochrome b_{-245} (Royer-Pokora *et al.*, 1986; Dinuer *et al.*, 1987; Teahan *et al.*, 1987). Phagocytic cells from most patients with autosomal recessive inheritance contain this cytochrome (Segal *et al.*, 1983), but it fails to receive electrons from NADPH when the cells are stimulated (Segal & Jones,

1980). The lesion was identified in these patients as a failure to phosphorylate a 47 kDa protein (Segal *et al.*, 1985; Heyworth & Segal, 1986), and these findings have now been confirmed in other laboratories (Kramer *et al.*, 1988; Okamura *et al.*, 1988).

The kinetics of activation of superoxide release by normal neutrophils corresponded to the kinetics of phosphorylation of this protein (Heyworth & Segal, 1986), and it was proposed that it is essential for the activation of the oxidase system, either as a component, or as a regulator, of the electron-transport chain. Consequently the nature and role of the 47 kDa phosphoprotein have become the focus of investigations into the mechanism of activation of this system.

We initially reported that the 47 kDa phosphoprotein is predominantly in the membrane fraction of neutrophils, with little present in the cytosol (Segal *et al.*, 1985). Hayakawa *et al.* (1986) and Kramer *et al.* (1988) have both referred to a dual localization in the cytosol and membrane fractions. In the present study we looked in more detail at the subcellular localization of the 47 kDa phosphoprotein in human neutrophils after stimulation with phorbol myristate acetate (PMA). As its absence in autosomal recessive CGD is associated with the failure of electron transport from NADPH to cytochrome b_{-245} , it is likely that the 47 kDa phosphoprotein integrates with the electron-transport chain of the oxidase. In X-chromosome-linked CGD the genetic lesion results in the absence of the 90 kDa subunit of cytochrome b_{-245} , but the 23 kDa subunit, encoded for by a different gene, is also missing (Parkos *et al.*, 1987; Segal, 1987). The larger component appears to play a structural role, namely holding the heterodimeric cytochrome in the membrane. We have therefore determined whether the absence of these molecules in

Abbreviations used: PMA, phorbol myristate acetate; CGD, chronic granulomatous disease; HBS, Hepes-buffered saline (full composition is given in the text); Tos-Lys-CH₂Cl, tosyl-lysylchloromethane ('TLCK').

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X-chromosome-linked CGD affects the distribution of the 47 kDa phosphoprotein in these patients' neutrophils.

EXPERIMENTAL PROCEDURES

Purification of cells and labelling with [32 P]P $_i$

Purification of neutrophils from freshly drawn human peripheral blood or from buffy-coat residues (South London Blood Transfusion Centre, London S.W.17, U.K.) and washing in Hepes-buffered saline (HBS; 136 mM-NaCl/2.7 mM-KCl/1 mM-MgSO $_4$ /1 mM-CaCl $_2$ /5.6 mM-glucose/20 mM-sodium Hepes, pH 7.4) were as previously described (Heyworth & Segal, 1986). Neutrophils (> 97% pure) were finally resuspended in pre-warmed HBS at a density of between 1×10^7 and 5×10^7 cells/ml and incubated at 37 °C for 1 h with carrier-free [32 P]P $_i$ (Amersham International) at 12.3 MBq/ml.

Cell activation and fractionation

[32 P]P $_i$ -labelled cells were stimulated with 162 nM-PMA for 2 min, except where noted. After the addition of 4 vol. of ice-cold HBS to the bulk of the suspension, the tubes were stored on ice for 10 min and centrifuged at 2 °C and 220 g for 5 min. The pelleted cells were washed once with ice-cold HBS and resuspended in breakage buffer consisting of 6% (w/w) (1.024 g/ml) sucrose, 1 mM-EDTA, 50 mM-NaF, 27 μ M-tosyl-lysylchloromethane (Tos-Lys-CH $_2$ Cl) 100 units of aprotinin (Trasylol; Bayer)/ml and 10 mM-Tris/HCl, pH 7.4, at a cell density of 1×10^8 /ml. The neutrophils were broken by two 10 s bursts of sonication at ~2 °C, separated by a 30 s cooling period, using the 3 mm probe of an MSE sonicator (at low power and amplitude 4). Approx. 95% of the cells were broken by this procedure.

Sonicated samples were loaded on to 8 ml of 15% (w/w) sucrose (1.063 g/ml) layered over 4 ml of 34% (w/w) sucrose, (1.153 g/ml) (see Garcia & Segal, 1984), with both sucrose solutions containing 20 mM-NaF, and EDTA, Tos-Lys-CH $_2$ Cl and aprotinin at the same concentrations as given above. After centrifugation of the sucrose gradients for 40 min at 2 °C and 195000 g (r_{av} . 11.46 cm) in a Kontron TST 41.14 swing-out rotor, the cytosol was removed from the top of the gradient, and the light membrane fraction from the 15/34% sucrose interface. The membranes were diluted 3-fold with a solution of 1 mM-EDTA, 50 mM-NaF, 27 μ M-Tos-Lys-CH $_2$ Cl and 100 units of aprotinin/ml and pelleted by centrifugation at 2 °C and 91000 g (r_{av} . 6.66 cm) for 30 min. Membrane and cytosol fractions were stored at -20 °C.

This method of fractionation is an adaptation of a procedure known to give good separation of cytosolic, membrane and granule components (Garcia & Segal, 1984). On the discontinuous gradients used here the azurophilic granules and the few remaining specific granules, with mean equilibrium densities of ~1.23 and ~1.19 g/ml respectively, were pelleted with nuclei and unbroken cells. Granule fractions were not routinely analysed as our preliminary studies, and a previous report (Hayakawa *et al.*, 1986) indicated that they contained very few phosphoproteins after 2 min stimulation with PMA. Specific granules degranulate under these conditions, and the granule membranes, containing cytochrome b_{-245} , fuse with the plasma membrane.

Electrophoresis and autoradiography

Membrane pellets, resuspended in the EDTA/NaF/Tos-Lys-CH $_2$ Cl/aprotinin solution, and cytosol were assayed for protein content by using the Pierce BCA reagent [Pierce (U.K.), Cambridge, U.K.] and bovine serum albumin as a standard. Samples of these fractions were diluted with electrophoresis sample buffer to give final concentrations of 2.5% (w/v) SDS, 4% (v/v) 2-mercaptoethanol, 1 mM-EDTA, 10% (w/v) sucrose, 0.01% (w/v) Bromophenol Blue, 0.01 M-Tris/HCl, pH 6.8, and a protein concentration of about 1.0 mg/ml. Where the protein concentration of the fraction was too low, as sometimes occurred with membrane prepared from small numbers of patients' cells, the protein was

Fig. 1. (a) Time course of phosphorylation of the 47 kDa protein and (b) the effect of varying the dose of PMA on the phosphorylation of the 47 kDa protein and 23 kDa subunit of cytochrome b_{-245}

Normal human neutrophils were prelabelled with [32 P]P $_i$, and for (a) stimulated with 162 nM-PMA for the times indicated and for (b) stimulated with various concentrations of PMA for 2 min. Cells were fractionated on sucrose gradients, and equal amounts of protein from the cytosolic and membrane fractions separated by SDS/polyacrylamide-gel electrophoresis. The dried gels were autoradiographed and scanned as described in the Experimental procedures section. Radioactivity associated with the cytosolic (●) and membrane-associated (▲) 47 kDa protein and the 23 kDa cytochrome subunit (■) was quantified by measuring the area under the peaks. The values, in arbitrary units, are the means of single determinations from either two or three experiments. In the latter case error bars are shown and represent the S.E.M.

Fig. 2. One-dimensional peptide mapping of the cytosolic and membrane-associated 47 kDa phosphoprotein

Samples of cytosol (C) and membrane (M) from [^{32}P]P $_i$ -labelled cells were subjected to SDS/polyacrylamide-gel electrophoresis. Bands of gel containing the 47 kDa phosphoprotein were excised, equilibrated in 0.125 M-Tris/HCl, pH 6.8, containing 0.1 % SDS, loaded into the wells of a 15 %-polyacrylamide gel and overlayed with the same solution containing 10 % (v/v) glycerol, 0.005 % (w/v) Bromophenol Blue and *S. aureus* V8 proteinase to give 0, 5, 50 and 500 ng/well. The proteins were run into the stacking gel, the power turned off for 30 min and the electrophoresis continued until the tracking dye reached the bottom of the gel. Scans of autoradiographs of the dried gel show the original 47 kDa phosphoproteins (arrowed) and the phosphopeptides generated at the three concentrations of proteinase.

precipitated with 10 % (w/v) trichloroacetic acid and resolubilized in the sample buffer to give the same protein concentration as above. This procedure did not change the patterns of protein staining or protein phosphorylation. All samples were immersed in a boiling-water bath for 4 min and subjected to SDS/polyacrylamide-gel electrophoresis, exactly as described by Heyworth & Segal (1986), using the Pharmacia low-molecular-mass calibration kit as marker proteins.

Coomassie Blue-stained gels were destained, dried under vacuum, autoradiographed with preflashed (Laskey & Mills, 1977) Amersham Hyper MP film bath for 4 min and subjected to SDS/polyacrylamide-gel electrophoresis, exactly as described by Heyworth & Segal (1986), using the Pharmacia low-molecular-mass calibration kit as marker proteins.

Phosphopeptide mapping

The generation and analysis of phosphopeptides from membrane-associated and cytosolic 47 kDa phosphoprotein was based on the method of Cleveland *et al.* (1977), using *Staphylococcus aureus* V8 proteinase and chymotrypsin (both from Boehringer Mannheim). Samples of membrane and cytosolic fractions, prepared as described above from [^{32}P]P $_i$ -labelled cells, were run on 0.75 mm-thick, 10 % (w/v)-acrylamide gels at 80 μg of protein/track. The gels were rapidly stained and destained, and a slice of gel was removed from each track at the position of the 47 kDa phosphoprotein. The remaining gel was dried and autoradiographed to check that no other phosphoprotein had been removed. Equili-

Fig. 3. Phosphoproteins in the cytosol and membrane fractions of normal and X-chromosome-linked-CGD neutrophils stimulated with PMA

Neutrophils from a normal control (N) and a patient with X-chromosome-linked CGD (X) were labelled with [32 P]P $_i$, stimulated with 162 nM-PMA for 2 min and fractionated on sucrose gradients as described in the Experimental procedures. Equal amounts of protein from cytosol (CYT) and membrane (MEM) fractions were separated by SDS/polyacrylamide-gel electrophoresis and the gel dried and autoradiographed. The positions and sizes of the marker proteins are shown, and the 47 kDa phosphoprotein and the 23 kDa cytochrome subunit are indicated by the arrows.

bration of the gel slices, loading into the wells of the second gel [0.75 mm thick, 15% (w/v) acrylamide, with a 5 cm stacking gel], addition of the proteinase and subsequent electrophoresis were exactly as described by Cleveland *et al.* (1977).

CGD patients and families

Four X-chromosome-linked CGD patients were studied, all of whom were cytochrome b_{-245} -negative and whose mothers were identified as heterozygote carriers. They were patients no. 3 and 4 described by Segal *et al.* (1983), and two others, aged 3 (S.C.) and 4 (I.G.) years. The heterozygote carriers studied were the mothers of patients no. 3 and S.C.

RESULTS

Fig. 1(a) shows the time course of phosphorylation of the 47 kDa protein. Samples of the membrane and cytosolic fractions at each time point were run on the same gel, with each track containing an equal amount of protein. Typical phosphoprotein patterns for the two fractions are shown in Fig. 3 (below). Phosphorylation of the cytosolic 47 kDa protein occurred within 10 s, reached a maximum at about 1 min, and slowly declined thereafter. The appearance of membrane-associated 47 kDa phosphoprotein occurred more slowly, with an apparent lag of between 10 and 30 s, but then continued to increase up to 3 min.

Dose-response curves for phosphorylation of the cytosolic and membrane-associated 47 kDa protein were very similar, with half-maximal effect occurring at ~ 30 nM-PMA (Fig. 1b). The dose-response curve for phosphorylation of the 23 kDa subunit of cytochrome b_{-245} is also shown. Preliminary results suggest that this polypeptide is in the plasma membrane and specific granules (Dinauer & Orkin, 1988), as would be expected from the known distribution of the haem signal of the cytochrome (Garcia & Segal, 1984).

To check that the cytosolic and membrane-associated 47 kDa phosphoproteins are indeed the same protein, or at least closely related, we subjected samples of each to one-dimensional peptide mapping according to the method of Cleveland *et al.* (1977). At each of three concentrations of *S. aureus* V8 proteinase, the pattern of phosphopeptides generated was identical, with digestion at the highest concentration of enzyme (500 ng of protein/well) producing two well-resolved phosphopeptides of apparent molecular mass 20 and 17 kDa (Fig. 2). With chymotrypsin, at 0.6 and 3.0 μ g/well, partial digestion of the phosphoprotein from membrane and cytosol fractions also produced identical phosphopeptide maps (results not shown).

The subcellular localization of the 47 kDa phosphoprotein in the X-chromosome-linked CGD patients and in the heterozygote carriers was very different from that seen in the normal controls. In all four X-chromosome linked CGD patients enhanced phosphorylation of the 47 kDa protein was undetectable in the membrane fraction and was confined to the cytosol (Fig. 3 and Table 1). In the heterozygote carriers a pattern intermediate between that seen in the patients and the normal controls or unaffected family members was observed (Table 1). The phosphorylated 23 kDa subunit of cytochrome b_{-245} was also clearly visible in the membrane fractions of normal neutrophils, its identity being confirmed by its absence from the membranes of all the patients with X-chromosome linked CGD (Fig. 3), but not those with autosomal recessive inheritance (Garcia & Segal, 1988).

A parallel decrease in phosphorylation of the 23 kDa subunit of the cytochrome and the membrane-associated 47 kDa protein, to about 30% of normal levels, was observed in the two heterozygote carriers (Table 1). The intensity of label in the cytosolic 47 kDa phosphoprotein was normal in these subjects. The decreased level of phosphorylation of the 23 kDa subunit of cytochrome b_{-245} presumably reflects the well-documented subnormal concentrations of cytochrome b_{-245} in some heterozygous X-chromosome linked carriers (Segal *et al.*, 1983).

The results shown in Table 1 are derived from gels in which approximately equal amounts of membrane and

Table 1. Phosphorylation of the 47 kDa protein and the 23 kDa subunit of cytochrome b_{-245} in normal, heterozygote carrier and X-chromosome linked-CGD subjects

Neutrophils were prelabelled with [32 P]P_i, stimulated with PMA for 2 min and fractionated on sucrose gradients. Samples of the cytosolic and membrane fractions containing equal amounts of protein were separated by SDS, and the dried gels were autoradiographed and scanned. Radioactivity associated with the 47 kDa and 23 kDa proteins was estimated by measuring the area under the peaks. Values are the mean $\pm$ S.E.M. (no. of determinations) in arbitrary units.

Subjects	Fraction . . . Protein . . .	Radioactivity (arbitrary units)		
		Cytosol	Membrane	
		47 kDa	47 kDa	23 kDa
Normal controls		22.75 $\pm$ 3.80 (4)	21.37 $\pm$ 2.00 (4)	14.12 $\pm$ 1.16 (4)
Heterozygote carriers		22.67 $\pm$ 5.99 (3)	5.20, 9.29 (2)	3.27, 5.03 (2)
X-chromosome-linked CGD patients		23.62 $\pm$ 5.10 (4)	1.75 $\pm$ 0.14 (4)	1.25 $\pm$ 0.32 (4)

cytosolic protein were run in each track. The total amount of protein in the cytosol is actually 10–15 times that in the membrane. There is therefore no apparent increase in the level of radioactivity associated with the cytosolic 47 kDa protein in X-chromosome linked CGD carriers and patients, corresponding to the apparent decrease in the membrane level.

DISCUSSION

We have confirmed the findings of Hayakawa *et al.* (1986) and Kramer *et al.* (1988) that the 47 kDa phosphoprotein is observed both in the cytosol and membrane fraction of normal neutrophils. Our initial observation that it is predominantly particulate (Segal *et al.*, 1985) was probably due to the relatively long stimulation time (10 min) used in that study, or to the selective action of protein phosphatases in the absence of fluoride from the breakage buffer.

The time course of appearance of the 47 kDa phosphoprotein at the two sites suggests that phosphorylation takes place in the cytosol and that the protein subsequently transfers to the membrane. Support for this concept is provided by peptide maps of the phosphoprotein from the two sources, which show an identical pattern of phosphopeptides, and by the very similar dose–response curves. The delay in the time course of membrane association of the 47 kDa phosphoprotein may be responsible for the lag between addition of PMA and the onset of superoxide generation.

In our initial description of the failure of phosphorylation of the 47 kDa protein in autosomal recessive CGD, cells from X-chromosome-linked CGD patients were used to demonstrate that the defect in phosphorylation did not simply result from the absence of NADPH oxidase activity. Andrews & Babior (1983) initially failed to find a phosphorylation defect in male and female CGD patients. Subsequently, by using two-dimensional electrophoresis, they described a partial defect, with phosphorylation of one of several 48 kDa phosphoproteins in male subjects with CGD (Hayakawa *et al.*, 1986). The genetic linkage in these patients was undefined, making it impossible to assess the implications of their results. Recently they have confirmed our findings with autosomal recessive patients and demonstrated that the

components of the 48 kDa phosphoprotein group, with different isoelectric points, are structurally very similar and probably the products of the same gene (Okamura *et al.*, 1988).

In the present study we have again demonstrated normal levels of phosphorylation of the 47 kDa protein in X-chromosome-linked CGD. These same patients have provided the basis for the important observation that, in the absence of cytochrome b_{-245} , the 47 kDa phosphoprotein apparently fails to transfer from the cytosol to the membrane fraction. The intermediate results with the heterozygote carriers, with a similar decrease in the levels of the membrane-associated 47 kDa phosphoprotein and the phosphorylated 23 kDa cytochrome b_{-245} subunit, suggest that the absence of this phosphoprotein from the membranes is stoichiometrically linked to the absence of the cytochrome b_{-245} subunits.

In a cell-free system it is possible to activate membrane-associated superoxide production with arachidonic acid or SDS, in the presence of cytosol (Bromberg & Pick, 1985; Curnutte, 1985; McPhail *et al.*, 1985). Reconstitution experiments have shown that the cytosolic factor is defective in autosomal recessive CGD and that superoxide generation (Curnutte *et al.*, 1988), and phosphorylation of the 47 kDa protein, in the cytosol and membrane (Caldwell *et al.*, 1988), are restored by normal cytosol. The cytosolic factor must therefore be physiologically important and, as it is unlikely that there are two genetic lesions, it is tempting to speculate that the 47 kDa protein and the cytosolic factor are the same component.

The possibility cannot be excluded that the 47 kDa protein has a permanent dual localization, but the observations of Caldwell *et al.* (1988) and the finding that membranes from cells primed with suboptimal doses of stimuli become less dependent on the presence of a cytosolic factor for superoxide generation (McPhail *et al.*, 1985), support the concept of stimulus-induced translocation.

If translocation of the 47 kDa phosphoprotein is required for PMA stimulation of the respiratory burst, it raises the question of what form of protein–membrane interaction is involved. Burn (1988) has suggested the possibility that in amphitropic proteins a conformational

change induced by phosphorylation could expose a lipophilic group, such as myristate or palmitate, leading to membrane insertion, in this case with the cytochrome presumably having a stabilizing role. Alternatively such a change could result in an increase in the protein's affinity for the cytosolic domain of one or both of the cytochrome subunits.

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REFERENCES

- Andrews, P. C. & Babior, B. M. (1983) *Blood* **61**, 333–340
- Bromberg, Y. & Pick, E. (1985) *J. Biol. Chem.* **260**, 13539–13545
- Burn, P. (1988) *Trends Biochem. Sci.* **13**, 79–83
- Caldwell, S. E., McCall, C. E., Hendricks, C. L., Leone, P. A., Bass, D. A. & McPhail, L. C. (1988) *J. Clin. Invest.* **81**, 1485–1496
- Cleveland, D. W., Fischer, S. G., Kirschner, M. W. & Laemmli, U. K. (1977) *J. Biol. Chem.* **252**, 1102–1106
- Curnutte, J. T. (1985) *J. Clin. Invest.* **75**, 1740–1743
- Curnutte, J. T., Berkow, R. L., Roberts, R. L., Shurin, S. B. & Scott, P. J. (1988) *J. Clin. Invest.* **81**, 606–610
- Dinauer, M. C. & Orkin, S. H. (1988) in *Hematology/Oncology Clinics of North America: Phagocytic Defects II: Abnormalities of the Respiratory Burst* (Curnutte, J. T., ed.), pp. 225–240, W. B. Saunders, Co., Philadelphia
- Dinauer, M. C., Orkin, S. H., Brown, R., Jesaitis, A. J. & Parkos, C. A. (1987) *Nature (London)* **327**, 717–720
- Garcia, R. C. & Segal, A. W. (1984) *Biochem. J.* **219**, 233–242
- Garcia, R. C. & Segal, A. W. (1988) *Biochem. J.* **252**, 901–904
- Hayakawa, T., Suzuki, K., Suzuki, S., Andrews, P. C. & Babior, B. M. (1986) *J. Biol. Chem.* **261**, 9109–9115
- Heyworth, P. G. & Segal, A. W. (1986) *Biochem. J.* **239**, 723–731
- Holmes, B., Page, A. R. & Good, R. A. (1967) *J. Clin. Invest.* **46**, 1422–1432
- Kramer, I. M., Verhoeven, A. J., van der Bend, R. L., Weening, R. S. & Roos, D. (1988) *J. Biol. Chem.* **263**, 2352–2357
- Laskey, R. A. & Mills, A. D. (1977) *FEBS Lett.* **82**, 314–316
- McPhail, L. C., Shirley, P. S., Clayton, C. C. & Snyderman, R. (1985) *J. Clin. Invest.* **75**, 1735–1739
- Okamura, N., Curnutte, J. T., Roberts, R. L. & Babior, B. M. (1988) *J. Biol. Chem.* **263**, 6777–6782
- Parkos, C. A., Allen, R. A., Cochrane, C. G. & Jesaitis, A. J. (1987) *J. Clin. Invest.* **80**, 732–742
- Royer-Pokora, B., Kunkel, L. M., Monaco, A. P., Goff, S. C., Newburger, P. E., Baehner, R. L., Cole, F. S., Curnutte, J. T. & Orkin, S. H. (1986) *Nature (London)* **322**, 32–38
- Schneider, C., Zanetti, M. & Romeo, D. (1981) *FEBS Lett.* **127**, 4–8
- Segal, A. W. (1987) *Nature (London)* **326**, 88–91
- Segal, A. W. & Jones, O. T. G. (1980) *FEBS Lett.* **110**, 111–114
- Segal, A. W., Cross, A. R., Garcia, R. C., Borregaard, N., Valerius, N. H., Soothill, J. F. & Jones, O. T. G. (1983) *N. Engl. J. Med.* **308**, 245–251
- Segal, A. W., Heyworth, P. G., Cockcroft, S. & Barrowman, M. M. (1985) *Nature (London)* **316**, 547–549
- Selvaraj, R. J. & Sbarra, A. J. (1966) *Nature (London)* **211**, 1272–1276
- Teahan, C., Rowe, P., Parker, P., Totty, N. & Segal, A. W. (1987) *Nature (London)* **327**, 720–721
- White, J. R., Huang, C.-K., Hill, J. M., Naccache, P. H., Becker, E. L. & Sha'afi, R. I. (1984) *J. Biol. Chem.* **259**, 8605–8611

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