Effects of lithium ions on actin polymerization in the presence of magnesium ions

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In spite of the abundant literature, questions on the biological action of Li^+ are far from being answered. In the present paper we demonstrate that modification of the salt composition of the medium for actin polymerization, by gradually replacing K^+ with Li^+ , leads to a dose-related change in the time course of actin assembly. The presence of Li^+ influences actin polymerization *in vitro* by enhancing nucleation and decreasing critical monomer concentration at steady state. Furthermore, Li^+ stabilizes actin polymers mainly by lowering the absolute value of the dissociation rate constant (K_d) and shifting (towards lower values of actin monomer concentrations) the range of G-actin concentrations in which filament-subunit flux can occur. The influence of Li^+ on actin and tubulin polymerization *in vitro* suggests that cytoskeletal structures could be some of the cytoplasmic targets of this ion.

INTRODUCTION

Actin filaments are present in muscle and non-muscle cells, and contribute to cell shape and motility manifestations such as cell locomotion and cytoplasmic spatial distribution of many organelles and macromolecules (Clarke & Spudich, 1977; Korn, 1978; Pollard, 1981; Couchman & Rees, 1982). To carry out its physiological role in non-muscle cells, actin continuously changes its aggregation state. The dynamic character of actin structures has been attributed to the ability of several proteins, small molecules and ions to interact with monomeric or polymeric actin and thereby to regulate actin assembly–disassembly by influencing the nucleation and elongation rates (Korn, 1982; Pollard & Craig, 1982; Stossel, 1989).

Actin assembly *in vitro* is usually induced by addition of neutral salts (e.g. 100 mM-KCl and/or 1–2 mM- MgCl_2). The mechanism of actin polymerization was investigated, with regard to ionic environment (Mg^{2+} , K^+ and Ca^{2+}), by Frieden (1982, 1983), Pardee & Spudich (1982), Maruyama & Tsukagoshi (1984) and Tellam (1985). More recently, Pan & Ware (1988) and Colombo *et al.* (1989) demonstrated that actin polymerization can be induced by LiCl alone, at concentrations ranging from 20 to 100 mM. Li^+ , under physiological conditions, is not present inside the cells, but it is commonly used as a psychotherapeutic agent (Cade, 1949; Birch, 1982; Johnson, 1984; Fieve *et al.*, 1985), in spite of the wide range of its undesirable side effects (Lazarus, 1986). Moreover, LiCl (30–300 mM) is teratogenic for the embryos of many organisms. Li^+ induces ‘vegetalization’ of embryos and disorganization of embryonic axes (Herbst, 1892; Nieuwkoop, 1970; Kao *et al.*, 1986; Breckenridge *et al.*, 1987).

Li^+ seems to substitute for K^+ inside the cell. Since its release from cells is very slow, it tends to be accumulated in cytoplasm at concentrations higher than in serum (Singer & Rotenberg, 1973). Therefore, we could foresee that, during Li^+ administration, the intracellular Li^+ concentration reaches values which are very close to those able to influence actin assembly.

Actin assembly in cells is mainly driven by Mg^{2+} ions, which occupy the high-affinity sites of actin monomers (Kitazawa *et al.*, 1982). Physiological concentrations of Mg^{2+} allow many characteristic phenomena, such as treadmilling or filament-subunit flux (Wegner, 1976; Neuhaus *et al.*, 1983) and F-actin fragmentation (Wegner, 1982; Wegner & Savko, 1982; Grazi & Trombetta,

1985), to occur. So we decided to extend the previous findings by Pan & Ware (1988) and Colombo *et al.* (1989) to show how the concomitant presence of Li^+ and Mg^{2+} could affect actin assembly and the actin-polymer characteristics.

MATERIALS AND METHODS

Actin from rabbit muscles was prepared by the procedure of Spudich & Watt (1971), modified by McLean-Fletcher & Pollard (1980). G-actin (M_r 42000; Collins & Elzinga, 1975) was stored on ice in buffer G (2 mM-Tris/HCl, pH 8, 0.2 mM-ATP, 0.5 mM-dithiothreitol, 0.2 mM- CaCl_2 , 1.5 mM- NaN_3) and used within 1 week. G-actin concentration was determined from the A_{290} by using an absorption coefficient of $0.617 \text{ mg}^{-1} \cdot \text{ml} \cdot \text{cm}^{-1}$ (Gordon *et al.*, 1976).

N-(1-Pyrenyl)iodoacetamide-labelled actin was prepared as previously reported (Colombo & Milzani, 1988). The pyrenyl-actin concentration and molar ratio were calculated from the A_{290} and A_{344} (Cooper *et al.*, 1983a; Kouyama & Mihashi, 1981).

Fluorescence measurements were carried out at 25 °C with a Kontron SFM 25 spectrofluorimeter, equipped with a neutral density filter (50 %) to avoid pyrenyl-actin photobleaching. To minimize light-scattering, a cut-off filter (which eliminates all wavelengths under 390 nm) was placed between the sample and the phototube (Grazi, 1985).

Gelsolin–actin complex was prepared from human platelets as reported by Selve & Wegner (1986). The concentration of the complex was determined by the methods of Lowry *et al.* (1951) and Bradford (1976).

Intermolecular cross-linking with *NN'*-1,4-phenylenebis-maleimide was performed during actin polymerization as described by Millonig *et al.* (1988).

SDS/PAGE (7.5 % gels) of cross-linked actin-polymerizing samples was carried out by the method of Laemmli (1970), and the gels were stained with Coomassie Brilliant Blue R-250.

Phalloidin-stabilized actin seeds were prepared by the method of Estes *et al.* (1981).

RESULTS

The fluorescent signal obtained from pyrenyl-actin is proportional to polymer concentration and is insensitive to filament

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Fig. 1. Influence of increasing LiCl concentrations on (a) the time course of actin polymerization and (b) the lag phase

(a) G-actin ($12\ \mu\text{M}$; containing 10% of pyrenyl-actin) was polymerized by salt addition, and the fluorescence intensity was measured with excitation at 365 nm and emission at 407 nm. The curves represent different time courses of actin polymerization induced by (from right to left): 2 mM- MgCl_2 + 100 mM-KCl; 2 mM- MgCl_2 + 90 mM-KCl + 10 mM-LiCl; 2 mM- MgCl_2 + 70 mM-KCl + 30 mM-LiCl; 2 mM- MgCl_2 + 50 mM-KCl + 50 mM-LiCl; 2 mM- MgCl_2 + 30 mM-KCl + 70 mM-LiCl; 2 mM- MgCl_2 + 10 mM-KCl + 90 mM-LiCl; 2 mM- MgCl_2 + 100 mM-LiCl. Panel (b) shows a detail from panel (a).

length distribution (Detmers *et al.*, 1981; Cooper *et al.*, 1983a). Therefore, to test actin assembly we have used such a fluorimetric technique.

In Fig. 1, time courses of fluorescence increases show that in 100 mM-LiCl + 2 mM- MgCl_2 actin polymerizes more efficiently than in similar samples in which LiCl was replaced by KCl. Fig. 1 also suggests that Li^+ acts by increasing the maximum rate of actin polymerization and shortening the lag phase, which is likely to include monomer activation and nucleation steps (Oosawa &

Asakura, 1975; Wegner & Engel, 1975; Tobacman & Korn, 1983; Cooper *et al.*, 1983b; Frieden & Goddette, 1983; Matsudaira *et al.*, 1987).

The influence of Li^+ ions on actin assembly is dose-dependent, i.e. it increases with increasing Li^+ concentrations and, furthermore, it is already appreciable at low Li^+ concentrations (10 mM).

Nucleation is the rate-limiting step of the polymerization process (Korn, 1985). It is thermodynamically unfavourable and involves the condensation of actin monomers to yield nuclei (Oosawa & Kasai, 1971).

We tested by intermolecular cross-linking the time-related oligomer pattern in polymerizing actin samples (Millonig *et al.*, 1988). This technique covalently binds molecular products of actin assembly together. Molecular components of a particular polymerizing actin sample will then become separable by SDS/PAGE, owing to their different M_r .

Fig. 2 clearly shows that the presence of Li^+ markedly enhances actin nuclei (upper-dimer) formation. Comparison of Figs. 2(a) and 2(b) shows that at 1.5 min after addition of 100 mM-LiCl + 2 mM- MgCl_2 , the low- M_r dimer has almost vanished, whereas the upper- M_r dimer and the heavier oligomers are already formed. In 100 mM-KCl + 2 mM- MgCl_2 , a similar pattern occurs only at 5 min after salt addition.

To understand the effect of Li^+ on the actin-elongation step, we investigated its influence on the growth of pre-formed stabilized actin seeds. When pre-formed F-actin (actin seeds) is added to a polymerizing actin sample, the increase in polymerization signal, at early times, is due only to the growth of added actin filaments. The lag phase completely disappears and the slope of the straight line related to the initial polymer increase is proportional to the elongation rate (Pollard, 1983).

Fig. 3 shows that Li^+ slightly increases the elongation rate with respect to that observed in actin samples in which LiCl was replaced by KCl. Thus nucleation seems to be the assembly step mainly influenced by Li^+ .

Furthermore, we tested the effect of Li^+ on actin-filament depolymerization. Depolymerization can be induced by diluting F-actin samples below the critical monomer concentration (C_c) or by lowering the salt concentration. Dilution experiments (Fig. 4) show that actin polymers, assembled in 100 mM-LiCl + 2 mM- MgCl_2 depolymerize more slowly than samples in which LiCl is replaced by KCl.

Finally, we measured the critical monomer concentration (C_c) of F-actin samples assembled in the presence (capped) or in the absence (uncapped) of gelsolin-actin complex (Fig. 5). G-actin (0.5 ml; $4\ \mu\text{M}$) was polymerized by 100 mM-LiCl + 2 mM- MgCl_2 or 100 mM-KCl + 2 mM- MgCl_2 , with or without 50 nM-actin-gelsolin complex. Capped or uncapped F-actin was used to nucleate the assembly of G-actin samples with increasing protein concentrations. Fig. 5 shows that the abscissa-intercept value of the line related to the uncapped $\text{Li}^+/\text{Mg}^{2+}$ samples is markedly lower than that of the uncapped $\text{K}^+/\text{Mg}^{2+}$ F-actin. A similar lowering occurs in the gelsolin-capped samples. Fig. 5 also shows that the lowering of C_c related to both capped and uncapped actin polymers, owing to Li^+ , causes the shift of the 'treadmilling range' towards lower monomer-concentration values. This fact suggests that, when actin assembly occurs in the presence of both Li^+ and Mg^{2+} (ionic conditions which are likely to occur in Li^+ -treated cells), actin filaments grow and shorten at free monomer concentrations lower than those related to microfilaments built at physiological salt conditions. According to the experiment reported in Fig. 4, the depolymerization constants (ordinate intercepts in Fig. 5) are markedly lower in the $\text{Li}^+/\text{Mg}^{2+}$ than in the $\text{K}^+/\text{Mg}^{2+}$ F-actin samples.

Finally, comparing the slopes of different straight lines (Fig. 5)

Fig. 2. Cross-linking during actin polymerization

G-actin samples ($24 \mu\text{M}$) were polymerized by: (a) $100 \text{ mM-KCl} + 2 \text{ mM-MgCl}_2$; (b) $100 \text{ mM-LiCl} + 2 \text{ mM-MgCl}_2$. At the times indicated below each electrophoresis lane, polymerizing samples were cross-linked with NN' -1,4-phenylenebismaleimide and analysed by SDS/PAGE. Abbreviations: M, monomer; LD, lower dimer actin aggregate with an apparent M_r of 86000; UD, upper dimer actin aggregate with an apparent M_r of 115000; HO, high- M_r oligomers. According to Millonig *et al.* (1988), under conditions where no polymers are formed (G-actin without addition of polymerizing salts) no oligomer cross-linking bands appeared (results not shown).

Fig. 3. Effect of Li^+ on actin-filament elongation

Phalloidin-stabilized F-actin seeds ($86 \mu\text{l}$; $2.4 \mu\text{M}$) were used to nucleate G-actin (3 ml ; $12 \mu\text{M}$, 10% pyrenyl-actin) assembly in the presence of MgCl_2 and KCl or LiCl: $\square$, $100 \text{ mM-KCl} + 2 \text{ mM-MgCl}_2$; $\blacksquare$, $100 \text{ mM-LiCl} + 2 \text{ mM-MgCl}_2$. Straight lines represent linear regressions calculated on the experimental fluorescence values at earlier times.

Fig. 4. Time-related depolymerization of actin polymers assembled at different ionic conditions

Gel-filtered G-actin samples ($24 \mu\text{M}$; 30% pyrenyl-actin) were polymerized at 25°C overnight, by addition of: 100 mM-KCl ($\circ$), 100 mM-LiCl ($\bullet$), 2 mM-MgCl_2 ($\triangle$), $100 \text{ mM-KCl} + 2 \text{ mM-MgCl}_2$ ($\square$) or $100 \text{ mM-LiCl} + 2 \text{ mM-MgCl}_2$ ($\blacksquare$). Steady-state F-actin samples were diluted 1:10 with buffer G and the decrease in fluorescence intensity was measured. The fluorescence of all steady-state F-actin samples was calibrated to 100 a.u.

we deduced that Li^+ does not appreciably affect the monomer association constant (according to the experiment reported in Fig. 3).

DISCUSSION

Physiological conditions for actin polymerization are $100 \text{ mM-KCl} + 2 \text{ mM-MgCl}_2$. Replacement of K^+ by Li^+ causes a dose-dependent decrease in the lag phase, with a parallel increase in the maximum polymerization rate. Li^+ effects are already appreciable at a LiCl/KCl concentration ratio of 1:9 (Fig. 1). Since the presence of Li^+ does not significantly affect the elongation rate (Fig. 3), the Li^+ effects on the time course of actin polymerization seem to be mainly caused by the marked decrease in the time of appearance of upper dimers (the smallest elongating nuclei, according to Millonig *et al.*, 1988) caused by this ion (Fig. 2).

F-actin polymerized in the presence of Li^+ showed a great stability, when diluted, compared with other differently assembled actin polymers, which depolymerize more quickly

(Fig. 4). It is noteworthy that Bhattacharyya & Wolff (1976) also observed a Li^+ -related polymer stability in microtubule suspensions.

Fig. 5 gives a more complete picture of the effects (caused by the Li^+/K^+ exchange in polymerizing salt mixtures) on actin polymerization. Using four different kinds of actin polymers (F-actin polymerized by $\text{KCl} + \text{MgCl}_2$ or $\text{LiCl} + \text{MgCl}_2$, each in both the presence and the absence of a barbed end-capping protein) and plotting the initial elongation rate of different polymers versus actin-monomer concentration, we obtained much interesting information.

In the presence of Mg^{2+} , Li^+ markedly lowers critical concentration values. This fact is appreciable in both the presence and the absence of barbed end-capping proteins. From the ordinate-intercept values, which are proportional to the dis-

Fig. 5. Dependence of the initial rate of fluorescence change on the actin monomer concentration in the presence of different polymerizing salts and capping

Small gel-filtered G-actin samples (0.5 ml; 4 μ M; 10% pyrenyl-actin) were polymerized by (□, ○) 100 mM-KCl + 2 mM-MgCl₂ or (■, ●) 100 mM-LiCl + 2 mM-MgCl₂ with (○, ●) or without (□, ■) 50 nM-gelsolin-actin complex. F-actin samples were then diluted (1:5, v/v) with G-actin samples (10% pyrenyl-actin) at increasing protein concentrations. The Figure shows the plots of the changes in the fluorescence intensity ΔF (arbitrary units)/min versus the concentration of added monomers. In this kind of experiment, the critical concentration values (C_c) formally correspond to the concentration of added G-actin at which no changes of fluorescence occur (abscissa intercepts). But we have to consider that F-actin samples (capped or uncapped), used to nucleate polymerization, also contain G-actin. So we corrected the abscissa-intercept values by the following equation:

$$C_c = C + (V_p/V_{tot})C_c$$

or

$$C_c = C + (V_p/V_{tot})C_c$$

where C_c is the real critical concentration, C is the concentration of added G-actin at which no fluorescence changes occur (abscissa intercept), V_{tot} is the total volume of the fluorescence sample, and V_p is the volume of F-actin sample used to nucleate polymerization. In this equation the assumption is made that steady-state F-actin samples contain actin monomers (C_c) which are diluted in the final samples by the factor V_p/V_{tot} . So the numerically correct critical-concentration values are: C_c uncapped_{Li/Mg} = 0.50 μ M, C_c uncapped_{K/Mg} = 0.87 μ M; C_c capped_{Li/Mg} = 1.37 μ M, C_c capped_{K/Mg} = 2.62 μ M.

sociation rate constant (K_-), we can argue that Li⁺-induced actin filaments have a K_- which is markedly lower than that of polymers grown under physiological salt conditions. This is in agreement with the low depolymerization rate showed by Li⁺-induced F-actin, during dilution experiments (Fig. 4).

Finally, comparing the slopes of different linear plots of elongation rate versus actin-monomer concentrations (which are proportional to the association rate constant, K_+), we can deduce that Li⁺ ions do not influence the association rate constant. This agrees with the results summarized in Fig. 3, showing a very moderate enhancing effect of Li⁺ on the elongation rate of stabilized actin seeds.

Treadmilling may play various important roles, ranging from transport of organelles to regulation of filament stability, in living cells (Kirschner, 1980; Wang, 1985). Considering the range of actin-monomer concentrations, enclosed between the critical

concentrations for capped or uncapped actin systems (assembled by identical salt mixtures), as the 'treadmilling range' (in which subunit flux can occur), it is clear that Li⁺ ions are able to shift this 'treadmilling range' towards lower actin-monomer concentrations. This fact suggests that Li⁺/Mg²⁺-induced actin filaments could grow or shorten at monomer concentrations lower than those suitable to obtain similar responses from physiologically assembled F-actin.

The biochemical results presented in this paper could acquire some physiological relevance, considering the following.

(1) Li⁺ enters the cell (Post, 1957; Smith & Rozengurt, 1978) by partly replacing intracellular K⁺ (Singer & Rotenberg, 1973). Therefore actin, in Li⁺-treated cells, is likely to polymerize in the presence of physiological concentrations of Mg²⁺ together with a mixture of K⁺/Li⁺, at undefined relative concentrations.

(2) Only F-actin is physiologically relevant for the cell (Korn *et al.*, 1987), so actin polymerization has a key role in cell motility.

(3) Different biochemical events (such as nucleation and treadmilling) characterizing the actin assembly and filament dynamics are finely tuned cellular mechanisms (Kirschner, 1980; Wang, 1985; Stossel, 1989). Also, small changes in their course could be very important for the correct development of cellular motility manifestations.

Many explanations for biological effects of Li⁺ have been suggested (Singer & Rotenberg, 1973; Rosenthal & Goodwin, 1982; Berridge *et al.*, 1989). Considering the results presented together with the data available in the literature, it seems to emerge that effects of Li⁺ on biological systems could also involve a direct influence of this ion on actin assembly in the cell.

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REFERENCES

- Berridge, M. J., Downes, C. P. & Hanley, M. R. (1989) *Cell* **59**, 411–419
- Bhattacharyya, B. & Wolff, J. (1976) *Biochem. Biophys. Res. Commun.* **73**, 383–390
- Birch, N. J. (1982) *Met. Ions Biol. Syst.* **14**, 257–313
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Breckenridge, L. J., Warren, R. L. & Warner, A. E. (1987) *Development* (Cambridge, U.K.) **99**, 353–370
- Cade, J. F. J. (1949) *Med. J. Aust.* **36**, 349–353
- Clarke, M. & Spudich, J. A. (1977) *Annu. Rev. Biochem.* **46**, 797–822
- Collins, J. H. & Elzinga, M. (1975) *J. Biol. Chem.* **250**, 5915–5920
- Colombo, R. & Milzani, A. (1988) *Biochim. Biophys. Acta* **968**, 9–16
- Colombo, R., Doglia, S., Giordano, P. & Milzani, A. (1989) *Springer Ser. Biophys.* **3**, 289–291
- Cooper, J. A., Walker, S. B. & Pollard, T. D. (1983a) *J. Muscle Res. Cell Motil.* **4**, 253–262
- Cooper, J. A., Buhle, E. L., Walker, S. B., Tsong, T. Y. & Pollard, T. D. (1983b) *Biochemistry* **22**, 2193–2202
- Couchman, J. R. & Rees, D. A. (1982) *Eur. J. Cell Biol.* **27**, 47–54
- Detmers, P., Weber, A., Elzinga, M. & Stephens, R. E. (1981) *J. Biol. Chem.* **256**, 99–105
- Estes, J. E., Selden, L. A. & Gershman, L. C. (1981) *Biochemistry* **20**, 708–712
- Fieve, R. R., Jamison, K. R. & Goodnik, P. J. (1985) *Neurol. Neurobiol.* **15**, 107–120
- Frieden, C. (1982) *J. Biol. Chem.* **257**, 2882–2886
- Frieden, C. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 6513–6517
- Frieden, C. & Goddette, D. W. (1983) *Biochemistry* **22**, 5836–5843
- Gordon, D. J., Yang, Y. Z. & Korn, E. D. (1976) *J. Biol. Chem.* **251**, 7474–7479
- Grazi, E. (1985) *Biochem. Biophys. Res. Commun.* **128**, 1058–1063
- Grazi, E. & Trombetta, G. (1985) *Biochem. J.* **232**, 297–300
- Herbst, C. (1892) *Z. Wiss. Zool.* **55**, 446–578
- Johnson, F. N. (1984) *Psychopharmacology of Lithium*, Oxford University Press, New York

- Kao, K. R., Masui, Y. & Elinson, R. P. (1986) *Nature (London)* **322**, 371–373
- Kirschner, M. W. (1980) *J. Cell Biol.* **86**, 330–334
- Kitazawa, T., Shuman, H. & Somlyo, A. P. (1982) *J. Muscle Res. Cell Motil.* **3**, 437–454
- Korn, E. D. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 588–599
- Korn, E. D. (1982) *Physiol. Rev.* **62**, 672–737
- Korn, E. D. (1985) *Curr. Top. Cell. Regul.* **26**, 221–232
- Korn, E. D., Carlier, M. F. & Pantaloni, D. (1987) *Science* **238**, 638–644
- Kouyama, T. & Mihashi, K. (1981) *Eur. J. Biochem.* **114**, 33–38
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Lazarus, J. H. (1986) *Endocrine and Metabolic Effects of Lithium*, Plenum Publishing Corp., New York.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Maruyama, K. & Tsukagoshi, K. (1984) *J. Biochem. (Tokyo)* **96**, 605–611
- Matsudaira, P., Bordas, J. & Koch, M. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3151–3155
- McLean-Fletcher, S. & Pollard, T. D. (1980) *Cell* **20**, 329–341
- Millonig, R., Salvo, H. & Aebi, U. (1988) *J. Cell Biol.* **106**, 785–796
- Neuhaus, J., Wanger, M., Keiser, T. & Wegner, A. (1983) *J. Muscle Res. Cell Motil.* **4**, 507–527
- Nieuwkoop, P. D. (1970) *Wilhelm Roux's Arch.* **166**, 105–123
- Oosawa, F. & Asakura, S. (1975) *Thermodynamics of the Polymerization of Proteins*, Academic Press, New York
- Oosawa, F. & Kasai, M. (1971) in *Subunits in Biological Systems* (Timasheff, S. & Fasman, G. D., eds.), part A, pp. 261–322, Marcel Dekker, New York
- Pan, X. X. & Ware, B. R. (1988) *Biophys. J.* **53**, 11–16
- Pardee, J. D. & Spudich, J. A. (1982) *J. Cell Biol.* **93**, 648–654
- Pollard, T. D. (1981) *J. Cell Biol.* **91**, 156–165
- Pollard, T. D. (1983) *Anal. Biochem.* **134**, 406–412
- Pollard, T. D. & Craig, S. W. (1982) *Trends Biochem. Sci.* **7**, 55–58
- Post, R. L. (1957) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **16**, 102
- Rosenthal, N. E. & Goodwin, F. K. (1982) *Annu. Rev. Med.* **33**, 555–568
- Selve, N. & Wegner, A. (1986) *Eur. J. Biochem.* **160**, 379–387
- Singer, I. & Rotenberg, D. (1973) *N. Engl. J. Med.* **289**, 254–260
- Smith, J. B. & Rozengurt, E. (1978) *J. Cell. Physiol.* **97**, 441–450
- Spudich, J. A. & Watt, S. (1971) *J. Biol. Chem.* **246**, 4866–4871
- Stossel, T. P. (1989) *J. Biol. Chem.* **264**, 18261–18264
- Tellam, R. (1985) *Biochemistry* **24**, 4455–4460
- Tobacman, L. S. & Korn, E. D. (1983) *J. Biol. Chem.* **258**, 3207–3214
- Wang, Y. L. (1985) *J. Cell Biol.* **101**, 597–602
- Wegner, A. (1976) *J. Mol. Biol.* **108**, 139–150
- Wegner, A. (1982) *Nature (London)* **296**, 266–277
- Wegner, A. & Engel, J. (1975) *Biophys. Chem.* **3**, 215–225
- Wegner, A. & Savko, P. (1982) *Biochemistry* **21**, 1909–1913

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