$^{54}\text{Mn}^{2+}$ as a tracer of the polymerization of actin

Intermediate oligomers condense to give F-actin

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(Received 7 February 1984/Accepted 29 June 1984)

Mg^{2+} , at submicromolar concentrations, is needed for the nucleation of actin [Maruyama (1981) *J. Biol. Chem.* **256**, 1060–1062]. I show here that Mn^{2+} fulfils the same function. It binds to oligomers present in the ATP–G-actin solutions with a ratio of 2–3 Mn^{2+} ions per 100 actin monomers and with an association constant of $0.66 \times 10^{10} \text{ M}^{-1}$ at pH 8.2 at 25°C. The time course of the binding of Mn^{2+} to polymerizing actin is not affected by the initial concentration of the protein. Analysis of the distribution of the binding shows that, both in the large oligomeric species and in the polymers, 1 Mn^{2+} ion is bound for every 14–25 actin monomers, whereas in the smaller oligomeric species 1 Mn^{2+} ion is bound for every 4 actin monomers. The proposal is made that Mn^{2+} stabilizes actin nuclei and decreases the concentration of the monomers at the steady state. It is also proposed that, at least in some experimental conditions, the direct condensation of oligomers of intermediate length is an effective mechanism of F-actin formation.

Maruyama (1981) has stressed the importance of the presence of submicromolar concentrations of Mg^{2+} (but not of Ca^{2+}) for the nucleation of actin. He has also found that nucleation occurs also in the absence of Mg^{2+} provided that the actin solution is kept under mechanical agitation.

Since the association constant of Mg^{2+} for the single ‘high-affinity site’ of actin is approx. $0.6 \times 10^5 \text{ M}^{-1}$ (Barany *et al.*, 1962; Strzelecka-Golaszewska, 1973), to explain the effect of submicromolar Mg^{2+} on the nucleation reaction it must be assumed that (a) either Mg^{2+} is bound to the ‘high-affinity site’ of a small fraction of the G-actin molecules that then undergo nucleation, (b) or Mg^{2+} is bound to a previously undetected ‘very-high-affinity site’ of the majority of the G-actin molecules, (c) or Mg^{2+} stabilizes preformed actin nuclei, as was proposed by Maruyama (1981).

Since, as shown in the present paper, Mn^{2+} can replace Mg^{2+} in promoting the nucleation of actin, it is possible to follow the formation of the $^{54}\text{Mn}^{2+}$ –actin complexes and to relate this process of binding to the polymerization of actin. As a result of these studies I show that $^{54}\text{Mn}^{2+}$ binds, with an association of $0.66 \times 10^{10} \text{ M}^{-1}$, to the oligomers (but not to the monomers) present in the G-actin solutions at $\eta_{\text{sp.}} = 0.00$. My studies suggest also that, in the course of the polymerization,

Mn^{2+} is bound to small oligomeric species (trimers to pentamers) and to larger oligomeric species (15 to 24 monomers). The latter are proposed to condense directly to give F-actin.

Materials and methods

ATP–G-actin (adenylate kinase-free) from rabbit muscle was prepared by the method of Spudich & Watt (1971). Occasionally purified G-actin was also submitted to Sephadex G-150 filtration in accordance with the procedure of McLean–Fletcher & Pollard (1980). Actin was stored as a concentrated solution (5 mg/ml) at 0°C in 0.2 mM-ATP/0.2 mM- CaCl_2 /0.5 mM-2-mercaptoethanol/0.5 mM- NaN_3 /2 mM-Tris/HCl buffer, pH 8.2. Actin concentration was measured from the absorbance at 290 nm, the absorbance of 1 mg of pure actin/ml (light-path 1 cm) being taken to be 0.62 (Gordon *et al.*, 1976). Alternatively, the Coomassie Blue method was used (Bradford, 1976). The molar concentration of G-actin was calculated on the basis of an M_r of 42000 (Collins & Elzinga, 1975).

Possible contamination with adenylate kinase was tested directly on 12 μM -actin solutions. Adenylate kinase activity was assayed in the presence of the coupled system consisting of phosphoenolpyruvate, pyruvate kinase, NADH

and lactate dehydrogenase by measuring ADP produced with ATP and AMP as the substrates.

Bovine pancreatic deoxyribonuclease I (grade II) was purchased from Boehringer, Mannheim, West Germany, and was found on polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate to be about 60% pure. Molar concentration of the enzyme solutions was calculated accordingly, on the basis of an M_r of 31 000. Deoxyribonuclease I solutions were dialysed for 3 h at 2°C against water before use.

Free Mn^{2+} and Ca^{2+} concentrations were calculated on a Tektronik 31 programmable calculator by using an iterative procedure. The following binding constants were used: EDTA- H^+ , 10.26; HEDTA- H^+ , 6.16; EDTA- Mn^{2+} , 14.0; EDTA- Ca^{2+} , 10.6 (Schwarzenbach & Ackerman, 1948a,b). ATP- H^+ , 6.9; ATP- Ca^{2+} , 5.0 (O'Sullivan & Perrin, 1961); ATP- Mn^{2+} , 5.0 (O'Sullivan & Cohn, 1966). EGTA- H^+ , 9.43; HEGTA- H^+ , 8.85; EGTA- Ca^{2+} , 10.7; EGTA- Mn^{2+} , 12.3 (Schmid & Reilley, 1957).

To avoid contamination with bivalent cations ATP stock solutions were treated with AG-50X8 (H^+ form) resin and then neutralized with aq. NH_3 .

EDTA, EGTA, $CaCl_2$ and $MnSO_4$ stock solutions were titrated by standard procedures.

Viscosity was measured with an Ostwald viscosimeter maintained at 25°C.

Sephadex G-50 filtration was performed at 25°C on columns (1.4 cm $\times$ 50 cm) equilibrated with Tris/HCl buffer at the concentration indicated in the single experiments. The flow rate was 4 ml/min. Fractions of volume 1 ml were collected. Each fraction was analysed for protein and for Mn^{2+} (radioactivity).

Results

Mn^{2+} replaces Mg^{2+} in promoting the nucleation of actin

Maruyama (1981) made the interesting observation that submicromolar concentrations of Mg^{2+} (but not of Ca^{2+}) are needed to stimulate the polymerization of actin. In Table 1 it is shown that Mn^{2+} can replace Mg^{2+} ; these cations, however, are required only at alkaline pH. At pH 6.6, polymerization of actin also takes place in the absence of the bivalent cation.

Binding of $^{54}Mn^{2+}$ in ATP-G-actin solutions

To determine the amount of $^{54}Mn^{2+}$ bound to actin, the protein, after 120 min of incubation in the presence of the cation, was isolated by filtration through Sephadex G-50 columns equilibrated with Tris/HCl buffer. In preliminary experiments it was shown that, after 120 min, the

Table 1. *Effect of submicromolar concentrations of either Mg^{2+} or Mn^{2+} on the polymerization of actin*

The incubation mixtures contained 26 μM -ATP-G-actin, 10 mM-ATP, 20 mM-Tris/HCl buffer, 10 mM-EDTA and 66 μM - Ca^{2+} (total concentration). At the beginning of the experiment either 2 mM- $MgCl_2$ or 2 mM- $MnCl_2$ (total concentrations) were added. pH was as indicated. Viscosity was measured after 60 min of incubation at 25°C.

Addition	Specific viscosity	
	pH 6.6	pH 8.0
None	0.74	0.00
Mg^{2+}	0.74	0.67
Mn^{2+}	0.70	0.44

Table 2. *Binding of $^{54}Mn^{2+}$ to G-actin as a function of the free Mn^{2+} concentration*

The incubation mixtures contained 23 μM -ATP-G-actin, 1 mM-ATP, 2 mM-Tris/HCl buffer, 50 μM - $^{54}MnCl_2$ (total concentration) (specific radioactivity 3800 c.p.m./nmol) and EGTA as indicated. The pH was 8.2, I was 0.011 and temperature was 25°C. After 2 h of incubation 0.5 ml of the reaction mixtures was submitted to gel filtration, as described in the Materials and methods section, on Sephadex G-50 columns equilibrated with 2 mM-Tris/HCl buffer, pH 8.2. The fractions collected were assayed for protein and for radioactivity. Actin, before the experiment, was dialysed against the 'actin buffer' from which Ca^{2+} was omitted.

Total EGTA (μM)	Free Mn^{2+} (nM)	Mn^{2+} ions bound per actin molecule
250	0.011	0.0026
150	0.024	0.0043
100	0.047	0.008
70	0.11	0.018
50	4.8	0.015
40	100	0.018
0	530	0.0188

binding of the cation was practically complete. EGTA was preferred to EDTA as the chelating agent because of its larger metal-ion buffering capacity in the range of free Mn^{2+} concentration between 0.01 and 1 nM. Essentially similar results, however, were also obtained with EDTA. As shown in Table 2, binding of Mn^{2+} to the protein becomes detectable at 0.01 nM free Mn^{2+} and reaches a plateau between 1 and 100 nM free Mn^{2+} . In contrast, binding decreases on raising the EGTA- Mn^{2+} -complex concentration. This shows that free Mn^{2+} is being bound and not the EGTA- Mn^{2+} complex. At 'saturation' about 2 Mn^{2+} ions are bound for every 100 molecules of actin (in some experiments up to 3 Mn^{2+} ions were bound).

Whatever the structures are that bind Mn^{2+} , their association constant for this cation is about $0.66 \times 10^{10} \text{ M}^{-1}$, i.e. more than 1000 times larger than the association constant for the single 'high-affinity site' of actin (Fig. 1). The possibility that $^{54}\text{Mn}^{2+}$ binds to protein contaminants of the actin preparation is unlikely because the same quantitative results are obtained both with the actin prepared by the method of Spudich & Watt (1971) and with the actin further purified by Sephadex G-150 filtration and an additional repolymerization-depolymerization cycle. The same conclusion is also supported by the finding that addition of either pancreatic deoxyribonuclease I or KI to the system decreases the binding of Mn^{2+} . In these experiments, which were performed essentially with the same conditions described in the legend of Table 2, $23 \mu\text{M}$ -ATP-G-actin was incubated for 60 min at 25°C with $0.1 \mu\text{M}$ - Mn^{2+} (free concentration) in the presence of either $58 \mu\text{M}$ -deoxyribonuclease I or 0.6 M -KI. The incubation mixtures were then submitted to gel filtration of Sephadex G-50 columns equilibrated with 2 mM -Tris/HCl buffer, $\text{pH} 8.2$. Analysis of the protein fractions showed that in the presence of either deoxyribonuclease I or KI the binding was decreased to 60% and to 35% respectively as compared with the control samples without deoxyribonuclease I or KI.

Binding of $^{54}\text{Mn}^{2+}$ to actin in the course of the polymerization: effect of the initial actin concentration

In these experiments actin polymerization in the presence of 0.032 nM - Mn^{2+} and 10 nM - Ca^{2+} (calculated concentrations) was compared with the

polymerization occurring in the presence of 52 nM - Ca^{2+} (calculated concentration). As shown in Fig. 2(b), $12 \mu\text{M}$ -actin fails to polymerize in the absence of Mn^{2+} , whereas in its presence it reaches an $\eta_{\text{sp.}}$ of 0.16 after 210 min of incubation; $24 \mu\text{M}$ - and $36 \mu\text{M}$ -actin solutions reached an $\eta_{\text{sp.}}$ of 0.66 and 0.7 respectively after incubation for 8 and 6 h in the presence of Mn^{2+} and about 14% of that value in the absence of Mn^{2+} .

The binding of Mn^{2+} to actin in the course of the polymerization was also followed, and it was found that it increases continuously during 8 h of incubation. At longer times actin begins to denature, and

Fig. 2. Binding of $^{54}\text{Mn}^{2+}$ to actin in the course of the polymerization: effect of the initial actin concentration

The incubation mixtures contained ATP-G-actin [$12 \mu\text{M}$ ($\square$) or $24 \mu\text{M}$ ($\circ$) or $36 \mu\text{M}$ ($\triangle$)], 4 mM -ATP, 0.5 mM -EDTA, 0.42 mM - $^{54}\text{MnCl}_2$ (specific radioactivity 4200 c.p.m./nmol), $60 \mu\text{M}$ - Ca^{2+} (including that initially bound to actin) and 40 mM -Tris/HCl buffer. The pH was 8.3, I was 0.06 and the temperature was 25°C . Control samples ($\blacksquare$, $\bullet$ and $\blacktriangle$) containing 0.48 mM - CaCl_2 (instead of $60 \mu\text{M}$ - Ca^{2+} and 0.42 mM - MnCl_2) were also prepared. At time intervals viscosity was measured on 1 ml samples of the incubation mixtures. Samples (0.5 ml) were also taken and submitted to gel filtration through Sephadex G-50 columns, equilibrated with 40 mM -Tris/HCl buffer, $\text{pH} 8.3$. The actin-containing fractions were then assayed for protein and for radioactivity as described in the Materials and methods section. (a) Mn^{2+} bound per actin monomer; (b) specific viscosity.

Fig. 1. Double-reciprocal plot of the binding of $^{54}\text{Mn}^{2+}$ to ATP-G-actin

Date are taken from Table 2.

the binding data are therefore less reliable. Of interest is the finding that (Fig. 2a) the time course of the binding of Mn^{2+} to actin is independent of the initial concentration of the protein. The value of the ratio Mn^{2+} ions bound/actin molecule, as a function of the time of incubation, appears, in fact, to lie approximately on the same curve independently of the initial concentration of actin.

Analysis of the partition, between the oligomeric and the polymeric species, of the Mn^{2+} bound to actin isolated by Sephadex G-50 filtration

In these experiments 27.6 μM -ATP-G-actin was incubated at 10.025 in the presence of 1 mM-ATP and 0.2 μM free Mn^{2+} (calculated concentration). As shown in Fig. 3, specific viscosity increases up to 0.7 in 215 min and then remains constant. Addition of 3 mM- $MgCl_2$ (final concentration) to the same samples, taken at different times of incubation, raises the specific viscosity to 1.2–1.3, independently of the length of the incubation in the presence of EGTA. This shows that in the time course of the experiment essentially no denaturation of the protein takes place. Even though the viscosity increase after 4–6 h of incubation is only 54–58% of that obtained by the further addition of 3 mM- $MgCl_2$, about 88% of the actin present in the medium is retained by a 0.22 μm -pore-size Millipore filter (Table 3) and thus it is in a highly polymerized state (Grazi & Magri, 1981).

Analysis of the distribution of $^{54}Mn^{2+}$ in the actin isolated by Sephadex G-50 filtration shows that, on average, 24% of the $^{54}Mn^{2+}$ appears in the Amicon-cone filtrate. Thus it represents the free $^{54}Mn^{2+}$ fraction in equilibrium with the Mn^{2+} -actin complexes. The remaining $^{54}Mn^{2+}$ is bound

to the protein and is distributed between the small oligomeric species (not retained by the Millipore filter) and the large oligomeric and polymeric species (retained by the Millipore filter) (Table 3).

The time course of the distribution between the two species is analysed in Fig. 4. For the polymeric species the ratio Mn^{2+} bound/total actin monomer varies between 0.041 and 0.069. This means that in

Fig. 3. Time course of the polymerization of actin at 10.025 in the presence of 0.2 μM free Mn^{2+}

The incubation mixtures contained ATP-G-actin (27.6 μM), 1 mM-ATP, 50 μM - $^{54}MnCl_2$ (specific radioactivity 4000 c.p.m./nmol), 30 μM -EGTA and 30 mM-Tris/HCl buffer. The pH was 8.2, I was 0.025 and the temperature was 25°C. At time intervals viscosity was measured on 1 ml samples (●). To the same samples 3 mM- $MgCl_2$ (final concentration) was then added and viscosity was measured again after 15 min of incubation (○). Actin, before the experiment, was dialysed against the 'actin buffer' from which Ca^{2+} was omitted.

Table 3. Partition of Mn^{2+} between the oligomeric and the polymeric species of actin

From the incubation mixtures described in Fig. 3 legend, at intervals, 0.5 ml samples were taken and submitted to filtration through Sephadex G-50 columns equilibrated with 30 mM-Tris/HCl buffer, pH 8.2. The fractions containing actin were pooled and subsequently divided into three equal portions. On the first portion (Sephadex G-50 filtrate) the protein and radioactivity were determined directly. On the second and third portions protein and radioactivity were determined on the effluent solutions obtained respectively by filtration either through 0.22 μm -pore-size Millipore filters (Millipore filtrate) or through Amicon CF50A cones (Amicon-cone filtrate), which have 95% retention for molecules with M_r above 50000. At long times of incubation the protein concentration falls because of tailing of F-actin on the column. Most probably tailing is due to purely mechanical reasons.

Time (min)	Sephadex G-50 filtrate		Millipore filtrate		Amicon-cone filtrate	
	Protein (μM)	Mn^{2+} (μM)	Protein (μM)	Mn^{2+} (μM)	Protein (μM)	Mn^{2+} (μM)
10	3.00	0.035	1.70	0.005	0.0	0.020
30	3.23	0.258	1.18	0.127	0.0	0.051
70	3.16	0.349	1.20	0.226	0.0	0.066
130	2.73	0.248	0.36	0.131	0.0	0.064
215	1.35	0.160	0.16	0.092	0.0	0.051
290	1.00	0.109	0.18	0.075	0.0	0.038
350	1.7	0.218	0.2	0.114	0.0	0.052

Fig. 4. Analysis of the distribution of $^{54}\text{Mn}^{2+}$ between the oligomers and the polymers of actin

Data are taken from Table 3. ●, $[\text{Mn}^{2+} (\mu\text{M})]$ in the Sephadex filtrate— $\text{Mn}^{2+} (\mu\text{M})$ in the Millipore filtrate]/[actin (μM) in the Sephadex filtrate—actin (μM) in the Millipore filtrate]; ○, $[\text{Mn}^{2+} (\mu\text{M})]$ in the Millipore filtrate— $\text{Mn}^{2+} (\mu\text{M})$ in the Amicon-cone filtrate]/[actin (μM) in the Millipore filtrate—actin (μM) in the Amicon-cone filtrate].

the polymers 1 Mn^{2+} ion is bound for every 14–25 monomers of actin. For the oligomeric species the ratio Mn^{2+} bound/total monomer of actin increases with the time of incubation. After 4–6 h it has the average value of 0.257. This means that, on average, 1 Mn^{2+} ion is bound for every 4 monomers of actin.

Discussion

I have confirmed the data of Maruyama (1981) that Mg^{2+} at submicromolar concentrations is needed for the polymerization of actin, albeit only at alkaline pH. I have shown further that Mn^{2+} can replace Mg^{2+} in this function.

At $1/0.011$ and η_{sp} , 0.00, 2–3 Mn^{2+} ions are bound per 100 actin monomers initially present in the solution. It is unlikely that this phenomenon is due to protein contaminants, as it is difficult to see how the binding of Mn^{2+} to contaminants could preserve actin from denaturation and become a necessary factor for polymerization. Nor is the finding explained by the binding of Mn^{2+} to an activated monomer (Cooper *et al.*, 1983), provided that this is not in exceedingly slow equilibrium with the non-activated monomers. Nor can actin dimers accept Mn^{2+} , since, in this case, the increase of the cation concentration from 1 to 100 nM would have greatly increased the concentration of the dimer– Mn^{2+} complex at equilibri-

um. The phenomenon, however, is explained by binding of Mn^{2+} to short oligomers in 'rapid' equilibrium with the monomers. This allows the formation of a limited amount of oligomer– Mn^{2+} complexes with the consequent decrease of the free monomer concentration that prevents further accumulation of oligomers. As an example, the effect of Mn^{2+} is given by a reaction scheme characterized by the association constant σK ($\sigma = 2 \times 10^{-7}$ and $K = 1.7 \times 10^{-5} \text{M}^{-1}$) for the rapid monomer–dimer equilibrium, and an association constant K for the formation of all the other oligomers (Wegner & Engel, 1975). At equilibrium total actin concentration will be given by:

$$[\text{a}]_{\text{total}} = [\text{a}] + 2[\text{a}_2] + 3[\text{a}_3] \dots + m[\text{a}_m] \quad (1)$$

$$[\text{a}]_{\text{total}} = [\text{a}] + \sigma K[\text{a}]^2 + 3\sigma K^2[\text{a}]^3 \dots + m\sigma K^{m-1}[\text{a}]^m \quad (2)$$

$$[\text{a}]_{\text{total}} = \sum_{j=2}^m j\sigma K^{j-1}[\text{a}]^j + [\text{a}] \quad (3)$$

Assuming for simplicity that Mn^{2+} binds only to the m -mers with an association constant $K_1 = 0.66 \times 10^{10} \text{M}^{-1}$, the concentration of the m -mer– Mn^{2+} complexes will be:

$$[\text{a}_m \text{Mn}^{2+}] = K_1[\text{Mn}^{2+}]\sigma K^{m-1}[\text{a}]^m \quad (4)$$

and thus at equilibrium and in the presence of Mn^{2+} :

$$[\text{a}]_{\text{total}} = \sum_{j=2}^m j\sigma K^{j-1}[\text{a}]^j + mK_1[\text{Mn}^{2+}]\sigma K^{m-1}[\text{a}]^m + [\text{a}] \quad (5)$$

where $[\text{a}]$ is the concentration of the monomers, $[\text{a}]_{\text{total}} = 23 \mu\text{M}$ is the sum of the concentrations of the monomers and of the monomers in the oligomers and $m = 20$. When Mn^{2+} concentration is increased from zero to 1 nM and to 100 nM, the concentration $[\text{a}]$ becomes 11.3 μM , 10.22 nM and 9.26 μM respectively, $[\text{a}_m]$ becomes 0.55 μM , 0.073 μM and 0.0011 μM respectively, and $[\text{a}_m \text{Mn}^{2+}]$ becomes 0 μM , 0.48 μM and 0.75 μM respectively.

Binding of Mn^{2+} to contaminants of the actin solution is also excluded by the effect of the addition of either deoxyribonuclease I or KI to the system.

In the presence of 0.1 μM – Mn^{2+} (free concentration), 58 μM –deoxyribonuclease I and 23 μM –ATP–G-actin, Mn^{2+} binding is decreased by 40% as compared with the binding in the samples without deoxyribonuclease I. This protein, which forms a 1:1 actin–deoxyribonuclease complex (Lazarides & Lindberg, 1974) with an association constant of

$5 \times 10^8 \text{ M}^{-1}$ (Mannherz *et al.*, 1980), thus competes with Mn^{2+} for actin and decreases the concentration of the Mn^{2+} -actin-oligomer complexes (association constant $0.66 \times 10^{10} \text{ M}^{-1}$).

In the presence of 0.6M-KI, which was reported to promote the depolymerization of actin (Szent-Györgyi & Szentkiralyi, 1960), binding of 0.1 μM - Mn^{2+} to actin is decreased by 65% as compared with the control samples without KI.

In conclusion, I propose that oligomers large at least as the trimer exist in the ATP-G-actin solutions, their formation involving at least 6–9% of the total monomers.

When the binding of Mn^{2+} to polymerizing actin is monitored, it is found that, in distinction to the time course of the polymerization, the time course of binding is practically independent of the initial actin concentration (12–36 μM). This could mean that, even though the oligomer population at different actin concentration may differ in size distribution, it reacts at similar rates with Mn^{2+} . It certainly means that the oligomer population is formed rapidly and extensively even at the lowest actin concentration tested. This is suggested also by the finding that the fraction of actin that is retained by the Millipore filter, and that represents the large oligomeric and polymeric species, increases more rapidly than viscosity. In this material the ratio Mn^{2+} bound/total actin monomers is practically constant over the whole time course of polymerization, so that both in the larger oligomers and in the polymers 1 Mn^{2+} ion is bound for every 14–25 actin monomers. These facts, taken together, suggest that aggregates composed of 14–25 monomers are formed rapidly and are then condensed into the long polymeric species. This proposal is also supported by evidence from electron microscopy: when actin polymerizes in 7.5mM-KCl (Ferri & Grazi, 1982; Grazi *et al.*, 1983) irregular aggregates (diameter 10–20 nm) are formed that then line up to form nascent filaments (Grazi *et al.*, 1984).

I have further shown that the ratio of the Mn^{2+} bound to actin not retained by the filter (small polymeric species) increases during the course of polymerization and finally reaches an average value of 1 Mn^{2+} ion bound for 4 monomers of actin. The value of their ratio falls in the range 3–5, which is the estimated size of the actin nuclei (Kasai *et al.*, 1960; Wegner & Engel, 1975; Wegner & Savko, 1982; Barden *et al.*, 1982; Tobackman & Korn, 1983; Cooper *et al.*, 1983). It seems therefore that the main function of Mn^{2+} at submicromolar concentrations is to stabilize the actin nuclei and to modulate the steady-state concentration of the monomer. The nuclei then grow to larger oligomers, which, at least in some conditions, condense into F-actin. This latter is the limiting

step of the overall process:

where the species marked by the star (*) are the actin- Mn^{2+} complexes; $3 < n < 5$ and $14 < m < 25$.

This work was supported by grants from the Italian Consiglio Nazionale delle Ricerche and from the Ministero della Pubblica Istruzione.

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