3H; H5), 6.96 (d, ^3J(H4,H3) = 7.8 Hz, 3H; H3), 7.39 (dd, ^3J(H3,H4) = 7.8 Hz, ^3J(H4,H5) = 7.8 Hz, 3H; H4). Elemental analysis (%) calcd for $C_{33}H_{51}N_7$: C 72.62, H 9.42, N 17.96; found: C 72.59, H 9.20, N 17.80.

1: A solution of PhCOONa (72 mg, 0.05 mmol) in H_2O (2.5 mL) was added to a solution of $Fe(ClO_4)_3 \cdot x\,H_2O$ (177.1 mg, 0.5 mmol) and tnpa (273.0 mg, 0.5 mmol) in acetonitrile (10 mL). After stirring for 1 h, the solution was concentrated to give a purple powder of **1**, which was collected by filtration, washed with a small amount of diethyl ether, and dried in vacuo (yield: 89%). Elemental analysis (%) calcd for $C_{40}H_{59}N_7O_8Cl_1Fe_1$: C 55.70, H 7.15, N 11.37; found: C 56.04, H 6.94, N 11.44.

2: $Fe(ClO_4)_3 \cdot x\,H_2O$ (177.1 mg, 0.5 mmol) was dissolved in methanol containing molecular sieves (3 Å). After 24 h, tnpa (273.0 mg, 0.5 mmol) was added to this solution. After stirring for 30 min, the mixture was concentrated to give a yellow-orange powder of **2**, which was collected by filtration and dried in vacuo (yield: 65%). Elemental analysis (%)calcd for $C_{35}H_{57}N_7O_6Cl_1Fe_1$: C 55.08, H 7.53, N 12.85; found: C, 54.90, H, 7.72, N, 12.72.

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A Photochemical Switch for Controlling Protein – Protein Interactions**

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Photocaged small molecules and proteins, molecules that can rapidly be converted from inactive into active form with light, have proven to be very useful tools in biology.[1] The ability to control protein – protein binding interactions would further extend this approach to a large number of cellular processes including signal transduction pathways, gene regulation, and protein trafficking. Chemical modification has been used to introduce photocleavable groups into proteins, but this method depends on a uniquely reactive residue being present on the protein surface in order to achieve high selectivity.[2] Here we use unnatural amino acid mutagenesis to photocage the interaction of the $p21^{ras}$ (ras) protein with its downstream effector p120 – GAP (GAP = GTPase-activating protein). The caged ras protein, in which Asp 38 is substituted with the *β-o*-nitrobenzyl ester of aspartic acid (Nb-Asp), retains its intrinsic GTPase activity but is unable to interact

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with p120–GAP until uncaged by irradiation with light at a wavelength of 355 nm. This approach should be applicable to the side chains of other amino acids that can be blocked with photocleavable protecting groups including serine, threonine, glutamate, and lysine. It may provide a general method for the study of signal transduction pathways and other biological processes, both in vitro and in vivo, in which specific protein interactions can be identified.

Mammalian proteins encoded by the *ras* genes function as key regulators in various signal transduction processes involved in cell growth and differentiation.[3] Activity is controlled by conformational changes that depend on bound guanine nucleotides: the ras–GTP complex (ras·GTP) adopts a conformation that is able to bind to, and interact with, downstream effectors, whereas the GDP-bound conformation of the ras protein is inactive.[4] The ratio of ras·GTP to ras·GDP is controlled by the protein's intrinsic GTPase activity, as well as by proteins that increase the dissociation of GDP from the complex (guanosine nucleotide release factors, GNRFs)[5] or accelerate the rate of GTP hydrolysis (GAPs).[6] In this capacity, p120–GAP is a negative regulator of ras function;[7] p120–GAP can also act as a downstream effector of ras, and hence as a positive regulator of ras function in some systems.[5, 8]

The interaction of ras and p120–GAP was caged by replacing Asp38 in the ras protein with the photocleavable Nb-Asp by unnatural amino acid mutagenesis.[9] Mutagenesis[10] and structural studies[11] have shown that amino acids 30–38 (loop 2 or switch I)[12] and amino acids 60–76 (loop 4 or switch II)[13] are critical for the ability of ras to interact with its effector proteins. In particular, the mutation of Asp38 to Ala prevents GTPase stimulation by p120–GAP and results in the loss of transforming capacity in NIH3T3 cells;[14] moreover, this mutation abrogates binding of ras to raf[15] and phosphitidylinositol-3 kinase[16] (other downstream effectors of ras) in vitro. In addition, the recent crystal structure of the ras–p120–GAP complex[11] shows the Asp38 side chain oriented towards the Lys949 residue of p120–GAP, thus forming a hydrogen-bonding interaction with this residue through a bound water molecule.[17]

The Nb-Asp was introduced into the ras protein by in vitro suppression of an Asp38→TAG amber mutant (amber codon = terminations codon UAG) using a chemically aminoacylated suppressor tRNA as previously described.[9, 18] Both wild-type (WT) and mutant proteins carry a shortened version of the *ras* gene in which the 18 carboxy-terminal amino acids are removed and replaced with a six-residue histidine tail to aid purification.[19] The aspartate residue was N-protected with allyl chloroformate and then converted into the bis-nitrobenzyl ester using dicyclohexylcarbodiimide, 4-dimethylaminopyridine (DMAP), and 1-hydroxy-1*H*-benzotriazole. Subsequently, the α-nitrobenzyl ester group was selectively hydrolyzed with lithium hydroxide.[20] The *N*-allyloxycarbonyl-β-nitrobenzyl aspartate was activated and coupled onto the dinucleotide 5′-phospho-2′-deoxyribocytidylriboadenosine (pdCpA), and the aminoacylated dinucleotide was ligated to the suppressor $tRNA_{CUA}$.[18] In vitro expression of the WT *ras* gene under control of the T7 promoter afforded about 20 $\mu g\,mL^{-1}$ of protein. The suppression efficiency for Nb-Asp acylated $tRNA_{CUA}$ at position 38 was approximately 60%. In vitro protein synthesis reactions containing the TAG mutant in the presence of the full-length unacylated suppressor $tRNA_{CUA}$ resulted in less than 1% of the ras protein as compared with reactions which contain $tRNA_{CUA}$ acylated with Nb-Asp.[21] This control reaction demonstrates that the preparation of the caged ras protein is largely free of contaminating protein generated by readthrough of the amber codon by nonspecific tRNAs. Both the WT and mutant proteins were purified on a nickel resin to greater than 95% purity with an overall yield of approximately 50%.[22]

Intrinsic GTPase activity was determined for both the WT and mutant proteins by measuring the rate of GTP hydrolysis in a single-turnover experiment using [γ-^{32}P]GTP.[23] The reaction products were separated by chromatography on polyethyleneimine and quantified by phosphorimaging. There is no significant difference in the rate of catalysis k_{cat} between the WT and mutant ras proteins.[24] Their intrinsic GTPase activities are unaffected by irradiation with light at a wavelength of 355 nm from a frequency-tripled neodymium-YAG laser (Table 1).[25] In contrast to the WT ras protein, the caged mutant shows no p120–GAP-mediated GTPase activity, again under single-turnover conditions in the presence of a 100-fold excess of p120–GAP.[26] These results show that the *o*-nitrobenzyl group efficiently blocks the productive association of ras and p120–GAP.

Table 1. Intrinsic and p120–GAP-mediated GTPase activities of wild-type and mutant ras proteins before and after irradiation.

Protein	k_{cat} [$s^{-1} \times 10^4$][a] (−GAP)	Specific activity [% GTP $min^{-1} \times 10^3$][b] (−GAP)	(+GAP)[c]	Ratio[d]
Nb-Asp[e]	1.6 ± 0.02%	7.6 ± 0.3%	8.0 ± 0.08%	1.1
Nb-Asp[f]	1.5 ± 0.2%	6.9 ± 0.6%	24 ± 2%	3.5
WT[e]	1.7 ± 0.1%	7.8 ± 0.2%	57 ± 0.6%	7.2
WT[f]	1.6 ± 0.4%	7.2 ± 1%	56 ± 3%	7.8

[a] Reference [22, 23]. [b] The activity was calculated as the initial rate of conversion of GTP into GDP. [c] Reference [25]. [d] Ratio of activities in the presence and absence of p120–GAP.[29] [e] Before irradiation. [f] After irradiation.

Upon irradiation, *o*-nitrobenzyl esters are known to undergo rapid photodecomposition to the free acid and nitrosobenzaldehyde.[27] Photolysis of the caged ras protein restored roughly 50% of the GAP-dependent GTPase activity relative to WT ras (Table 1).[25, 26] This conversion was also monitored by denaturing polyacrylamide gel electrophoresis of the [^{35}S]methionine-labeled protein (Figure 1). The caged mutant has slightly lower electrophoretic mobility than the WT protein. After photolysis to remove the *o*-nitrobenzyl ester group, approximately 50% of the mutant protein is converted into product with the same mobility as the WT ras protein. The similarity in the levels of the restored p120–GAP-mediated GTPase activity and the conversion into a product with WT ras protein gel mobility upon photolysis suggest that the uncaged protein has WT p120–GAP-dependent GTPase activity. Photolysis of the caged mutant for longer times did not result in greater conversion into the

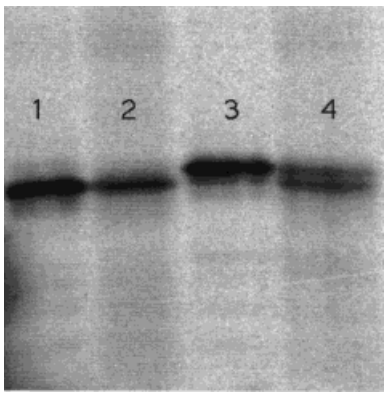

Figure 1. Autoradiogram of SDS polyacrylamide gel (16%) after purification and before and after irradiation of the [^{35}S]methionine-labeled protein products of in vitro translation reactions containing WT ras and Nb-Asp mutant. The caged protein (lane 3) has slightly lower electrophoretic mobility than the WT protein (lane 1). After photolysis to remove the *o*-nitrobenzyl ester group, about 50% of the mutant has shifted electrophoretic mobility (lane 4), while the mobility of the WT protein (lane 2) remains unaffected.

WT ras protein. This may be a consequence of a side reaction of the Nb-Asp with amino acids (300 μM) present in the in vitro synthesis reaction, which results in the formation of an inactive amide side chain. Alternatively, ras may be modified by the *o*-nitrosobenzaldehyde by-product of the photolysis reaction.[28]

In conclusion, these results show that installing Nb-Asp at position 38 of a ras protein generates a photocaged protein that retains full intrinsic catalytic activity, but is incapable of participating in signal transduction through its effector p120–GAP. After irradiation of the mutant, the WT protein is regenerated and its ability to interact with its effector protein is restored.

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10 mM ethylenediamine tetraacetate (EDTA), 1 mM 1,4-dithiothreitol (DTT), and 1% bovine serum albumen (BSA) at 4 °C in a volume of 400 μL. After 30 minutes this exchange reaction was stopped by the addition of $MgCl_2$ to give a final concentration of 25 mM and the enzyme · substrate (E · S) complex purified on a PD10 column. The purified E · S complex was divided into separate aliquots of 150 μL, and rGTP was added to give a final concentration of 50 μM. The reactions were incubated at 30 °C for various times before mixing 7.5-μL samples with 7.5 μL of stop solution (100 μM rGTP, 100 μM rGDP, 250 mM EDTA, 0.5% sodium dodecasulfate (SDS)). Conversion of GTP into GDP was monitored by chromatography on polyethyleneimine (0.3 M KH_2PO_4, 1 M $LiCl_2$, pH 3.5).

[24] For k_{cat} calculations based on monitoring the single-turnover experiment of the E · S complex in the presence of an excess of free unlabeled rGTP, the following relationship holds: $[P]/[E \cdot S]_0 = k_{cat}/(k_{cat} + k_{off}) \exp[-(k_{cat} + k_{off})t]$, where [P] is the concentration of the GDP product, t is time, and k_{off} the off-rate for GTP in the E · S complex. This relationship is based on the following assumptions: The E · S complex can break down only to product or to free enzyme and free substrate; the observed reaction is strictly single-turnover, because once the labeled substrate has dissociated from the enzyme it will not appreciably reassociate because of competition by excess unlabeled substrate; and the product off-rate is not limiting because the E · P complex is broken down in the stop reaction.

[25] Both WT and photocaged proteins were irradiated for up to five minutes with a frequency-tripled Spectra-Physics model GCR-150-10 laser (ca. 100 mJ at 355 nm, nominal pulse width of 7 ns). Longer photolysis times did not lead to greater conversion. Irradiation was performed in quartz cuvettes with stirring to ensure an even exposure. Reactions were chilled to 4 °C prior to irradiation to prevent overheating. The 500-μL reactions contained 10 nM WT or mutant ras protein in 50 mM Hepes (pH 7.5), 1 mM $MgCl_2$, 200 mM NaCl, 1 mM DTT, 0.1% BSA, and 10 μM semicarbazide · HCl to scavange the nitrosobenzaldehyde by-product.

[26] The p120 – GAP assay was performed by isolation of the E · S complex as described in ref. [23], except that a greater than 100-fold excess of p120 – GAP over the ras protein was added after dividing the E · S complex into separate reactions. p120 – GAP was a gift from H. Chung and was prepared by overexpression from a derivative of pUC18 carrying the gene for bovine p120 – GAP behind the Trp promoter as described in M. S. Marshall, W. S. Hill, A. S. Ng, U. S. Vogel, M. D. Schaber, E. Scolnick, R. A. F. Dixon, I. S. Sigal, J. B. Gibbs, *EMBO J.* **1989**, *8*, 1105 – 1110.

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[28] Improved deprotection efficiencies have been reported when the *o*-nitrobenzyl group is substituted at the α-benzylic position because the photocleavage by-product of these compounds is the less reactive nitrosoketone. Attempts to improve the efficiency of the photocleavage reaction by using β-1-(3,4-methylenedioxy-6-nitrobenzyl)-ethyl aspartate resulted in lower yields of mutant protein.

[29] Under saturating conditions, p120 – GAP can catalyze the GTPase rate of ras by 10^5-fold;[30] however, catalysis in these experiments is limited by the ras protein, which is present in the reaction at a concentration of about 10 nM but has a k_d for p120 – GAP of about 17 μM.[31]

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One- and Two-Dimensional Electron Transfer Processes in Triarylamines with Multiple Redox Centers**

Christoph Lambert* and Gilbert Nöll

Intramolecular electron transfer (ET) processes have been studied extensively in inorganic binuclear transition metal complexes;[1] organic donor – acceptor systems that are bridged by σ frameworks have been investigated in order to mimic biological ET processes.[2] In contrast, much less is known about purely organic Robin/Day[3] class II compounds with bridging π-electron systems and the electron transfer processes involved: Nelsen et al.[4] studied a number of bis(hydrazine)phenylene radical cations and related species; Bonvoisin et al.[5] investigated *m*-phenylene-bridged triarylamine systems. These radical cations are characterized by intervalence charge-transfer (IV-CT) bands in the NIR spectra which are associated with a photoinduced intramolecular electron transfer between two redox centers. As the coupling of redox centers over π-conjugated bridges is of central interest for the design of new optoelectronic materials,[6] it was our aim to study basic intramolecular electron transfer processes in simple symmetrically substituted π systems. We chose π-bridged triarylamine derivatives since triarylamines in general are important hole carrier systems, which are widely used in organic optoelectronic devices.[7]

4,4′-Bis(*N,N*-di-*p*-methoxyphenylamino)tolane (**2**) was synthesized by two Hagihara cross-coupling steps, and the corresponding trimer hexakis[4-(*N,N*-di-*p*-methoxyphenylamino)phenyl]benzene (**3**) was formed from **2** by catalysis with $[Co_2(CO)_8]$ (Scheme 1).[8] These derivatives should exhibit an intramolecular degenerate electron transfer in the singly and triply oxidized forms, respectively. Both neutral compounds are yellow, and their UV/Vis spectra in CH_2Cl_2 contain a strong absorption band at 373 nm (**2**) and 308 nm (**3**, with a shoulder at 330 nm). The cyclic voltammogram (CV)[9] of **2** shows two waves at 200 and 340 mV (versus Fc/Fc^+) in CH_2Cl_2 (Figure 1) referring to two reversible oxidations. This corresponds to a comproportionation constant ($K_{co} = 10^{(\Delta E/0.059)}$, where ΔE is the difference of redox potentials for the two oxidative steps) of 236 and suggests **2** to be a class II derivative.[1, 3] In contrast, trimer **3** only shows one broad unresolved wave centered at 225 mV. This reversible oxidation process was calibrated by thin-layer coulometry with 2,5-bis(dicyanovinyl)furan as the reference, and it involves six electrons in total, leading to $\mathbf{3}^{6+}$.

UV/Vis/NIR spectroelectrochemical investigation[10] of **2** shows that besides a band at 734 nm, which is characteristic for triarylamine radical cations,[11] a second very intense band

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